ENDOTHELIAL MEDIATION OF CORONARY VASCULAR TONE: NITRIC OXIDE ATTENUATION OF CHOLINERGIC VASOSPASTIC CHALLENGE

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ABSTRACT

Title of Dissertation: Endothelial Mediation of Coronary Vascular Tone: Nitric oxide attenuation of cholinergic vasospastic challenge

Neil W. Ahle, Doctor of Philosophy, 1993

Dissertation Directed by: Jack E. McKenzie, Associate Professor; Department of Physiology

The hypothesis tested was that the endothelium modulates the effect of acetylcholine, a vasoconstrictor of porcine coronary arteries, and limits the loss of flow occurring during a cholinergic vasospastic episode. Acetylcholine was injected into a coronary artery in an open-chest swine model before (ACh only) and after infusion of substance P (SP+ACh), an endothelial-dependent vasodilator, and nitroglycerin (NTG+ACh), an endothelial-independent vasodilator in two groups, Control and N^{ω}-nitro-L-arginine (NOLA) treated. NOLA is a chemical inhibitor of an endothelium-derived relaxing factor (EDRF) thought to be nitric oxide (NO) produced by vascular endothelium. The hemodynamic parameters of mean and phasic coronary artery flows, arterial and intraventricular pressures, dP/dt and ECG Lead II were continuously recorded and compared across the groups to account for changes attributable to the endothelium. Prior to conducting the experimenal study the presence of an endothelial role was validated by testing the conducting segment of a coronary artery with acetylcholine before and after mechanical denudation of its endothelium. Non-denuded areas were non-reactive to acetylcholine while denuded areas decreased in diameter from 14.32mm to

9.15mm, a net reduction in cross-sectional area of 59%. Acetylcholine produced initial dose-dependent decreases in coronary blood flow, immediately followed by a dose-dependent hyperemia. Slopes of best-fit lines to mean values during trough and hyperemic phases were -1.76 and 1.53, respectively. Both NTG and SP attenuated the loss of flow during cholinergic vasospasm and decreased the hyperemia that followed in the control group. Trough and hyperemia slopes were -1.68 and 0.64 during NTG treatment and -1.45 and 1.35 during infusion of SP. In addition, trough and hyperemia flows during EDRF blockade by NOLA were respectively lower and higher than flows during the corresponding Control period. The NOLA-treated Trough slopes for the ACh only, NTG+ACh and SP+ACh experiments were -2.51, -1.88 and -1.91, respectively; hyperemia slopes were 5.42, 3.76 and 3.63 for the same groupings. These findings validate the endothelial source of NO and its response to cholinergic vasospastic challenges, and add to the body of literature concerned with endothelial functions and mediation of coronary flow.

ENDOTHELIAL MEDIATION OF CORONARY VASCULAR TONE:

Nitric oxide attenuation of cholinergic vasospastic challenge

by

Neil W. Ahle, D.V.M.

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requirements for the degree of
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This work is dedicated to my children Matthew, Justine, and Jeffrey. Of the few small things I have been able to accomplish, or dream of attaining in the future, they are without doubt the only that will last or matter when I look back upon a lifetime's achievement.

A final note to my readers:

"You can find in a text whatever you bring, if you will stand between it and the mirror of your imagination. You may not see your ears, but they will be there."

Mark Twain, *A Fable*, 1909

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LIST OF ABBREVIATIONS

ACh	Acetylcholine
ACh only	Experiments using only ACh DRC
ANOVA	Analysis of Variance
CA	Coronary Artery
CVIS	Coronary Vascular Imaging System
CVR	Coronary Vascular Resistance
dP/dt	First derivative of Pressure (change in pressure over time)
DRC	Dose Response Curve
D.V.M.	Doctor of Veterinary Medicine
ECG	Electrocardiogram
EDCF	Endothelium-derived Contracting Factor
EDRF	Endothelium-derived Relaxing Factor
Fr	French
HA	Hyperemia Area
HD	Hyperemia Delta
HR	Heart Rate
IM	Intramuscular
IV	Intravenous
LADCA	Left Anterior Descending Coronary Artery
LCFXCA	Left Circumflex Coronary Artery
L-NAME	N^G-nitro-L-arginine methyl ester
L-NMMA	N^G-monomethyl-L-arginine
L-NOARG	L-N^G-nitro arginine
MAP	Mean Arterial Pressure
NO	Nitric Oxide
NOLA	N^{ω}-nitro-L-arginine
NTG	Nitroglycerin
NTG+ACh	Experiments receiving ACh DRC during NTG infusion
PIP	Peak Intraventricular Pressure
PPCF	Peak Phasic Coronary Flow
PRP	Pressure-Rate Product
PTCA	Percutaneous Transluminal Coronary Angioplasty
SP	Substance P
SP+ACh	Experiments receiving ACh DRC during SP infusion
TA	Trough Area
TD	Trough Delta
VSM	Vascular Smooth Muscle

SIGNIFICANCE

The role of the endothelium in mediating vascular tone has been studied extensively since Furchgott and Zawadski's seminal discovery in 1980 of an endogenous vasodilator they termed endothelium-derived relaxing factor (EDRF)[38]. Since then the list of compounds that act directly on the endothelium or through it and elicit a vascular response has grown. Lüscher and Vanhoutte[62] have listed over thirty distinct stimuli that release EDRF (as well as another thirteen shown to produce EDCF, or endothelium-derived contracting factor) in addition to the local and neurohumoral factors that regulate endothelium-dependent responses. Ignarro *et al.*[49] and Palmer *et al.*[83] have established the identity of EDRF as nitric oxide (NO), the active moiety of nitrovasodilator drugs used in clinical medicine. Nitrovasodilators can be viewed as prodrugs in the production of NO and include organic nitrates and nitrites, S-nitrosothiols, and sodium nitroprusside[27]. The validation of EDRF as NO forms a 'common ground' between the endogenous production of NO by the endothelial cell and its exogenous use in clinical medicine.

The loss of or damage to the endothelial cell surface, whether through aging, diet or iatrogenic intervention, has been shown to adversely affect the nature and quality of the endothelial response to a stimulus. Species differences appear to play a major role in variation of responses seen by a number of investigators, although when acetylcholine (ACh) is applied to damaged segments the response uniformly appears to be a loss of the expected relaxation or outright

vasoconstriction. Cox *et al.*[22] found decreased relaxation in acutely denuded dog coronary arteries (CA) tested with ACh *in vitro*, while Schipke *et al.*[88] saw relaxation reverse to contraction in the denuded epicardial coronary arteries of anesthetized dogs *in vivo*. Application of ACh to atherosclerotic human[61] and denuded porcine[42] CA segments caused contraction of the affected segment; the same porcine work reported the effect of ACh to be independent of the endothelium. ACh was chosen as a vascular constrictor for this study because although Cowan and McKenzie[21] were able to definitively demonstrate porcine coronary artery vasoconstriction *in vivo*, they were not able to find a parasympathetic component regulating basal coronary vascular tone. This study was designed to: 1) assess endothelial ability to modulate coronary vascular spasm through the release of endogenous nitric oxide, and 2) compare the effect of endogenously released NO to exogenous sources of it.

Acetylcholine Effect on the Endothelium and Vascular Tone

EDRF-driven vasodilation in response to ACh was proven in rabbit heart[2] and aorta[33,38] and dog femoral artery[3,33], leading to the assumption that ACh caused a uniform dose-dependent vasodilation. Species comparisons done *in vitro* by Kalsner[53] showed that although rabbit aorta and dog CA did in fact dilate in response to ACh, coronary arteries from sheep, pig and cattle hearts always contracted even in the confirmed presence of endothelium. Work done by Knight

et al.[56] in conscious dogs agreed with these and other dog studies[19,28,75,96], although the results in conscious calves and tranquilized baboons from the same study were more in line with Kalsner's non-dog species. In the calves, left circumflex coronary artery (LCFXCA) diameter initially fell but then rose significantly immediately following ACh, indicative of the trough and peak of a vasoconstrictive/hyperemic event. The baboons also demonstrated this biphasic response at high doses of ACh but exhibited only an increase in coronary blood flow and a decrease in coronary resistance at very low doses. Kalsner's study also examined strip and ring preparations of human CA which contracted when exposed to both low and high concentrations of ACh. This response was found to be blocked by atropine.

Human CA vasoconstriction in response to ACh has been validated by other investigators in non-atherosclerotic preparations[7,32,40,95]. Some investigators precontracted CA segments and searched for the expected vasodilation that was seen in the dog[7,41] but were able to report only vasoconstriction. Two quantitative angiographic studies done *in vivo*[73,77] did report vasodilation of a human CA by ACh. Nabel *et al.*[73] positioned a Doppler flow catheter in the proximal portion of either the left anterior descending (LAD) or LCFX CA and found a significant increase in both flow and calculated cross-sectional area in response to ACh. Newman *et al.*[77] performed his experiment in a similar fashion on the LAD but examined the proximal, middle, and distal portions of the vessel. A similar (although insignificant) dose-dependent dilation of the proximal segment was reported; the middle and

distal segments responded the same at low ($<10^{-3}$M) doses but constricted (a net lumenal diameter decrease) at high ($\geq 10^{-3}$M) doses. The decrease in diameter of the distal segment was described as "more marked" than the middle segment. The conclusion of Newman *et al.* was that location of the coronary vascular segment under consideration may have a significant effect on the observed response.

The pig was found to have similar responses to ACh administration when directly compared to the human CA in two *in vitro* studies[42,53]. Gräser *et al.*[42] reported both human and porcine CA contraction by ACh, and the reaction of both species was independent of the presence of endothelium. Kalsner[53] had also demonstrated only contraction in response to ACh in both species. One study using an *in vivo* pig model agreed closely with Newman's findings. McKenzie *et al.*[66] demonstrated profound ACh-induced vasoconstriction in distal (resistance) portions of the LADCA but elicited no response in proximal (conductance) segments. This is significant because an initial objective of this study is to validate the presence of an endothelial role in vessel modulation. Testing with ACh before and after mechanical removal of conductance segment endothelium will refute or validate the endothelium's ability to regulate vasospastic episodes.

The pig has proven to be a reliable research model that most closely approximates man in coronary size and vasculature[47] and has been used for coronary research in a number of studies[5,21,41,52,74,76]. Cowan's work with the *in vivo* pig CA showed that reductions in flow and conductance occur following intracoronary ACh[21] while other studies concentrated on the endothelium[5,41] or

vasospastic effects[52,74,76]. The release of EDRF from pig CA by ACh has been verified and quantified by Christie *et al.* using an *in vitro* cascade system[15], and a number of other works have documented endogenous porcine CA EDRF/NO release as a result of other stimuli[9,12,14,15].

Figure 1 diagrams some of the known actions of ACh, NTG and substance P at the vascular endothelial cell-smooth muscle cell interface. The muscarinic receptor subtypes responsible for denuded porcine CA contraction have been identified as M3 and M4; these are a logical candidate as the source of the direct vasoconstrictive action of ACh on the vascular smooth muscle (VSM) cell[98]. The mechanism of action of NTG, NO and substance P, as well as studies that defined NO as the EDRF, are detailed in the next section.

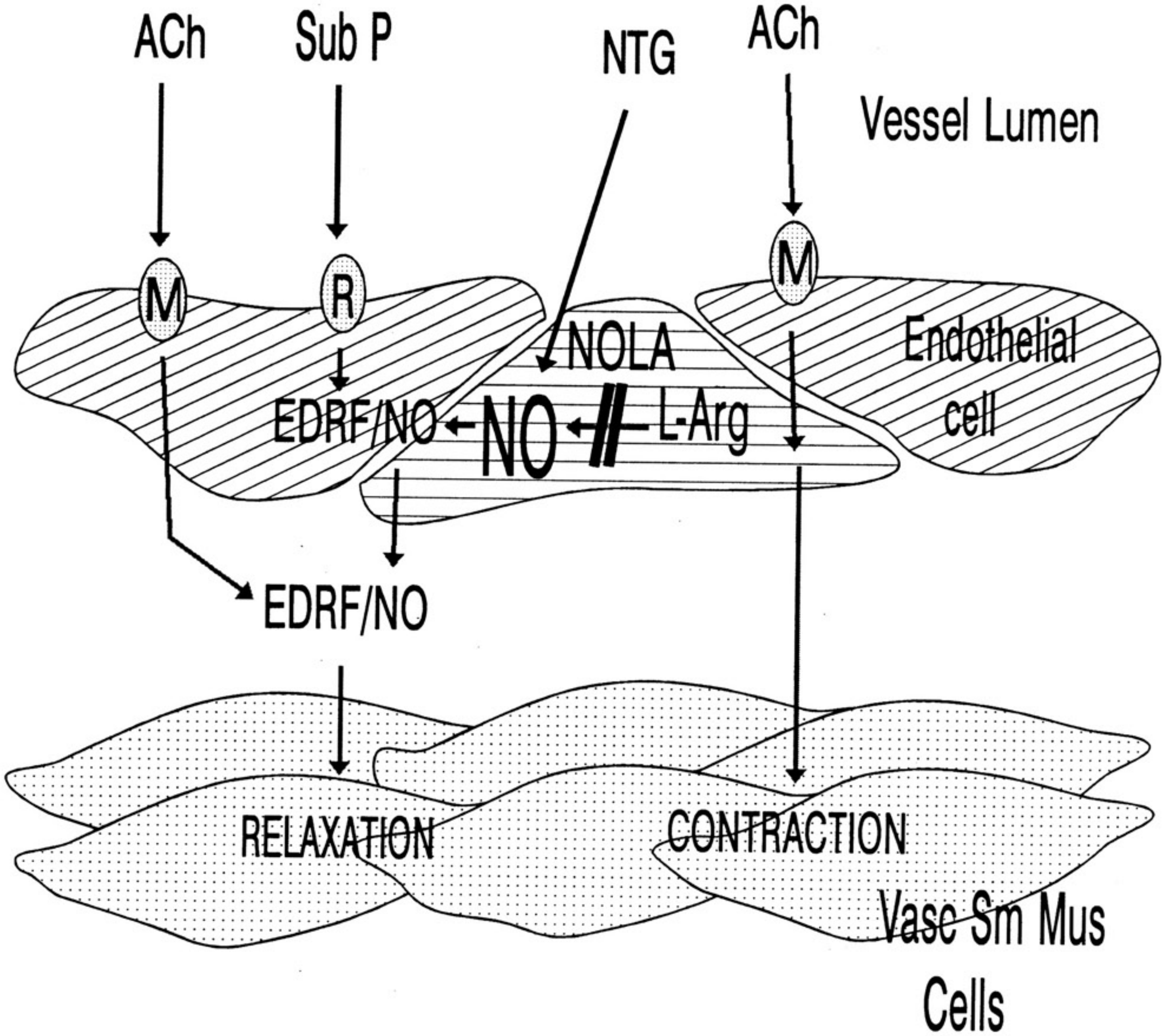

Figure 1. Actions of nitroglycerin (NTG) and substance P (Sub P) as nitric oxide (NO) generators at the endothelial cell/vascular smooth muscle cell interface. ACh = acetylcholine, EDRF = Endothelial Derived Relaxing Factor, L-Arg = ℓ-arginine, M = muscarinic receptor, R = substance P receptor. The heavy slashed lines indicate N^{ω}-nitro-L-arginine (NOLA) blockade of NO formation from L-Arg.

Nitric Oxide Sources or Generators

Endogenous

As stated in the opening paragraph, Furchgott and Zawadzki[38] were the first to note that although ACh was a potent vasodilator *in vivo*, graded contractions could be produced *in vitro* if the endothelial surface was carefully abraded away. They attributed the difference to the intact endothelium of the *in vivo* study and the release of substance(s) from it following stimulation of muscarinic receptors on the endothelial surface by ACh. Their proposed substance was termed endothelium-derived relaxing factor[37] and DeMey *et al.*[25] later raised the possibility of more than one EDRF. Förstermann *et al.*[34] has reported differences between species in the nature of EDRF as well.

Porcine aortic endothelial cell cultures have provided data on both the nature of EDRF and the method of its release[9,87]. The work of Boulanger *et al.*[9] demonstrated the existence of two EDRF-like substances, one released basally (tonically) and another under stimulation by bradykinin, while Rubanyi and Vanhoutte[87] showed a transient and a sustained component, differentially inhibited, that also suggested more than one EDRF-like substance. Ignarro *et al.*[49,50] and Palmer *et al.*[83] have established that at least one of these factors is nitric oxide (NO), a conclusion that is supported by Buga *et al.*[11] and Chaudhuri *et al.*[13] in bioassay experiments.

Palmer *et al.* have shown that 1) endothelial cells in culture synthesize NO from the amino acid L-arginine, and that 2) the reaction is specific because analogues of L-arginine, including its D- enantiomer, are not utilized as substrates[82]. The evidence for the chemical nature of EDRF as NO has been reviewed extensively by Moncada *et al.*[69,70], but two studies that provide direct comparisons of EDRF and NO are noteworthy. Palmer *et al.*[83] quantified the release of NO from porcine aortic cells in culture and found that concentrations of bradykinin capable of releasing EDRF also caused a concentration-dependent release of NO. Hutchinson[48] compared the pharmacologic profile of EDRF and exogenous NO on vascular strips and found that EDRF and NO were potentiated or inhibited in a like manner by the same compounds.

The mechanism of action of NO is well established and was summarized in the review of Moncada's *et al.*[70]. Early work demonstrated that organic nitrates increased cyclic guanosine monophosphate (cGMP) in smooth muscle, and it was subsequently determined that all nitrovasodilators as well as NO activate soluble guanylate cyclase. It has been established that activation of this enzyme leads to increases in cGMP levels, inducing a sequence of protein phosphorylation associated with smooth muscle relaxation[70].

Of the many substances known to cause endothelium-dependent dilations, one has been in use for decades[61]. Substance P (SP), a tachykinin, has been used in numerous studies[3,15,16,24,26,36,44,64] requiring a known endothelium-dependent vasodilator. Gulati *et al.*[43] characterized the tachykinin receptors on the pig CA *in*

vitro and many analogies to human CA's were seen. A previous *in vivo* pig study concluded that SP is a potentially important modulator of coronary blood flow, dilating the CA's and increasing coronary blood flow while causing systemic hypotension[26]. Other *in vivo* studies using SP in the canine intestine[35,86], rat stomach[46], and goat rumen[99] examined the stimulatory (spasmogenic) effect of intraarterial SP on the musculature. Although SP was found to have potent contractive effects in all associated musculature[35,46,86,99], it was not sufficient to overcome the potent vasodilatory action produced[65,86]. The action of SP may be mediated by cholinergic interneurones since atropine reduces its effects[35,46]. The dual stimulatory and spasmogenic effects may account for the finding that, in the rat, SP at low doses is a vasodilator but increases systemic vascular resistance at higher doses[44]. Removal of the endothelium has been found to remove dilator response to SP without affecting the dilatory ability of the organic nitrates[3].

Exogenous

Nitroglycerin (NTG) is probably the best known of the organic nitrates because of its long use in coronary medicine. It has been shown to preferentially dilate the large coronary arteries including collateral and epicardial conductance arteries[18,100], and Forman and Kirk were able to demonstrate a greater and more prolonged reduction in large vessel resistance compared with small vessel resistance following NTG injection[30]. A review of nitrovasodilator pharmacology illustrates that the group's vasodilatory ability arises from either spontaneous

cleavage to NO or metabolism to it by enzymatic and nonenzymatic processes; NTG requires free thiols such as N-acetylcysteine and the formation of a thionitrate as the intermediate[27]. A comparison of nitrovasodilators on isolated dog coronary arteries by Ohba's *et al.*[79] supports this, demonstrating that incubation with N-acetylcysteine potentiated the relaxant effects of NTG and reduced the development of tolerance to it. Schipke *et al.*[88] have demonstrated that endothelial denudation does not alter the reactivity of the vessel to NTG, at least acutely in anesthetized dogs.

Removal of Nitric Oxide Sources

Mechanical

The *ex vivo* removal of endothelium is a straightforward process that involves the mechanical abrasion of the intimal surface, usually using moist gauze sponges or other semi-abrasive materials[3,57,63,97]. Removal of endothelial NO sources *in vivo* is more difficult both in method and survivability of the model. To effectively accomplish it, investigators have used arterial embolectomy catheters[54,90], angioplasty or wedge pressure balloon catheters[93], extracorporeal distilled water perfusion[88] or even a bronchial lavage brush[22]. The techniques of porcine CA endothelial denudation and the validity of the model have been established. Shimokawa *et al.*[89-91] have found that a 2F Fogarty Embolectomy Catheter inflated with saline and withdrawn up the LAD five times will successfully remove

endothelium, while Steele *et al.*[93] used a polyethylene balloon catheter also inflated and withdrawn five times to denude carotid arteries.

<u>Chemical</u>

The work of Palmer's *et al.* with L-arginine analogues[84] showed that N^G-monomethyl-L-arginine (L-NMMA) inhibited synthesis of NO in a dose-dependent and isomer-specific manner. More studies of the L-arginine analogues with vasoactive properties have since been done, including L-NMMA[17,20,39,85], L-N^G-nitro arginine (L-NOARG or L-NNA)[71,72], N^G-nitro-L-arginine methyl ester (L-NAME)[39,85] and N^ω-nitro-L-arginine (NOLA)[17,101]. All of the L-arginine analogues have vasoconstrictive properties, produce dose-dependent hypertension, and act through the inhibition of the NO-forming enzyme, NO synthase[68]. L-NMMA increased arteriolar but not venous tone in humans[20] and produced constriction in skeletal muscle microvessels in rats[39] and rabbits[85]. When L-NOARG and L-NMMA were compared within the same study, both inhibited vasodilator effects of ACh on rabbit aorta and rat mesentery in a concentration-related manner[71]. Comparison of L-NAME to L-NMMA indicated that L-NAME had similar although less profound effects on increasing blood pressure in conscious rat[39] and anesthetized rabbit[85] studies. NOLA was found to be more potent than L-NMMA in blocking vasoactive effects of ACh over the same concentration range in isolated dog coronary artery[17] and anesthetized rat[101] studies.

Changes in Reactivity Due to Endothelial Alterations

Post-denudation

Changes in vessel reactivity are commonly seen after injury to the endothelium. The vasospastic nature of denuded vessels *in vitro* and the effect of vasoactive agonists has been detailed in the preceeding sections. *In vivo* denudation, on the other hand, has most often been performed in order to establish a chronic lesion that would lead to atherosclerotic plaque development. A few acute endothelial denudation studies have reported responses similar to that of the *in vitro* work. One acute study by Schipke *et al.*[88] using anesthetized dogs demonstrated epicardial vasoconstriction and an increase in epicardial resistance as a response to ACh in distilled water denuded LCFX coronary arteries. Lam *et al.*[58] directly correlated vasoconstriction with platelet deposition in pigs sampled immediately following balloon angioplasty; Steele *et al.*[93] noted that platelet deposition was heaviest 24 hours post-angioplasty but markedly reduced 4 days post. Cox *et al.* reported loss of c-GMP mediated relaxations in canine coronary arteries following "acute endothelial denudation" although he considered five weeks post-denudation to be an acute time period[22]. This study should be evaluated carefully since Shimokawa *et al.* have observed that "histologically, atherosclerotic changes were predominant along the denuded portion of the LCX" one to three months post-denudation[90] in pigs fed a 2% cholesterol laboratory chow post-

denudation. The majority of *in vivo* studies found in the literature are directed toward the creation and/or evaluation of atherosclerotic lesions.

Atherosclerosis

In vitro studies involving human atherosclerotic lesions have reported a reduction of endothelium-mediated vasodilation[8,10,31] and c-GMP formation[8]. Berkenboom *et al.* examined isolated, precontracted human coronary arteries and noted no response to the endothelium-dependent vasodilator substance P when moderate to severe atherosclerosis was present[6]. *In vivo* work has been done both with laboratory animal and human subjects. Shimokawa's work with Göttingen miniature swine reported enhanced vasospastic activity in 1 to 3 month post-denudation atherosclerotic lesions challenged with histamine[89-91]. Fischell *et al.*[29] have documented acute, progressive coronary artery vasoconstriction at and distal to the site of percutaneous transluminal coronary angioplasty (PTCA), one of the current therapies for reduction of atherosclerotic occlusions. LaVeau *et al.*[59] have reported that in atherosclerotic rabbit femoral arteries, segments proximal and distal to the site of balloon angioplasty were highly reactive and could contribute to vessel closure 28 days post-angioplasty. In summary, the presence of atherosclerosis has been found to severely reduce the ability of affected vessels to limit vasospasm arising from several different stimuli.

RATIONALE

The endothelium is known to contribute significantly in the mediation of coronary vascular dynamics, especially in the regulation of coronary blood flow. Products manufactured and released by the coronary endothelial cells act on the underlying vascular smooth muscle, selectively dilating or constricting it in accordance with the nature of the stimulus. The purpose of this study was to test the endothelium's ability to modulate the response to a specific vasospastic cholinergic challenge by acetylcholine.

Two test groups were examined: one group received a bolus dose of N^{ω}-nitro-L-Arginine (NOLA) which inhibits synthesis of EDRF and another group acted as control. The hypothesis tested was that in response to a cholinergic challenge the coronary endothelium acts to modulate the effects of the vascular spasm and maintain flow to the myocardium. The following aspects of endothelial/vascular wall interactions were examined for each group: 1). Dose response curves to ACh boluses were established (ACh only), 2). Endothelial-dependent vasodilation by SP infusion was begun and followed by ACh bolus (SP+ACh) and 3). Nonendothelial-dependent vasodilation by NTG infusion was begun and followed by ACh bolus (NTG+ACh).

The specific aims to be accomplished based on these tests were:

1. To determine the direct effects on coronary blood flow of cholinergic challenge to normal and EDRF-neutralized coronary endothelium.

2. To determine the ability of endothelial-dependent and -independent vasodilators to counter the cholinergic effects on normal and EDRF-neutralized endothelium.

EXPERIMENTAL DESIGN AND METHODS

Experimental Design

Endothelial mediation of cholinergic mechanisms governing coronary vascular tone was evaluated by comparing the effects of three vasoactive substances (ACh, SP, and NTG) in two studies (Control and NOLA-blocked). The effect of a dose-response curve (DRC) of randomized intracoronary bolus doses of normal saline and 0.5, 1.0, 2.0, 3.0 and 5.0 μg ACh (acetylcholine chloride, Sigma Chemical Company) was determined (ACh only). The DRC was repeated during endothelium-dependent coronary artery vasodilation by SP (SP+ACh), and again during endothelium-independent vasodilation by NTG (NTG+ACh). Intracoronary infusion rates for NTG (ICI Americas) and SP (Peninsula Laboratories) were 10 µg/min and 7.4 pmole/sec, respectively. The time period between ACh bolus doses was sufficient to allow coronary flows to return to a stable baseline prior to the next dose being administered. Experimental trials requiring blockade of EDRF were randomly selected to receive a one-time 4 mg/kg body weight IV bolus dose of NOLA (Sigma Chemical Company). Other studies using one of the arginine analogues *in vivo* have used one-time IV bolus doses of 30 mg/kg L-NMMA in the dog[55] and the rat[92] and 10 mg/kg L-NAME in the rat[94]. A lower dosage was chosen for this study since NOLA has been found to be approximately 70 times more potent

than L-NMMA as an inhibitor of EDRF release[51]. The efficacy of EDRF blockade is recognized by a rise in mean arterial pressure (MAP),[1] and the 4 mg/kg dose used here was sufficient to elevate MAP an average of 8.6 ± 0.96 mm Hg for the five animal NOLA group.

MAP measured at the aortic root, peak intraventricular pressure (PIP), the change in intraventricular pressure with time (dP/dt), LADCA and LCFXCA mean and phasic blood flows, and ECG Lead II were continuously recorded on the Gould recorder as well as on digital electronic media (Computer, Model Z-284, Zenith Data Systems, Inc.) by a custom-made data collection program (Branch Technology). While this study focused on changes in LADCA flow seen after ACh injection, these other data were collected as indicators of pan-cardiac effects. Changes in dP/dt, PIP and LCFXCA flows in particular demonstrate an ACh-induced effect on cardiac mechanics indicative of a non-localized (and thus undesirable) response. Arterial and coronary sinus blood gas samples were withdrawn into iced heparinized syringes for determination of pO_2, pCO_2, pH and O_2 content. At the conclusion of the experiment the animals were euthanized with an overdose of the anesthetic agent or KCl simultaneous with injection of a high-carbon ink into the LADCA catheter. This procedure stained that portion of the myocardium perfused by the LADCA, allowing it to be excised and weighed as an estimation of the mass of tissue perfused. Calculations were then done to normalize flow per 100 grams of tissue perfused.

Prior to conducting the experimental study, the presence of an endothelial role in the maintenance of coronary vascular tone was validated by testing the conductance section of the LADCA with ACh before and after mechanical denudation of its endothelium.

Preparation and Testing of Denuded Animals

Healthy domestic Yorkshire/Hampshire cross female or castrated male swine weighing between 15 and 25 kg were sedated with 15 mg/kg Ketamine hydrochloride (Vetalar®, Fort Dodge Laboratories) intramuscular (IM), weighed, and anesthetized with Thiopental sodium (Pentothal®, Abbott Laboratories) to effect through a marginal ear vein catheter. Following endotracheal intubation the animals were placed in dorsal recumbency on a V-tray, clipped and scrubbed ventrally on the neck, and draped to prepare an aseptic field for surgery.

Using aseptic technique a ventral midline incision was made and the left carotid artery isolated. Blood flow in the artery was stopped and a portion of the vessel brought exterior using two Lig-a-Loops® (Axiom Medical) passed around its circumference and then retracted. A 16 gauge, 2 inch Angiocath® (Intravenous Catheter Placement Unit, The Deseret Company) was inserted into the lumen of the artery (but not past the proximal Lig-a-Loop® that occluded blood flow) and the stylet removed, leaving the cannula in place. The guide wire of a 9 or 10 French (Fr) catheter sheath (Catheter Sheath Introducer System, Cordis Corporation) was

inserted through the bore of the cannula, and the cannula removed. The remainder of the catheter sheath system (vessel dilator and catheter sheath) was assembled and advanced over the guide wire into the carotid artery, at which time the guide wire and dilator were removed from the sheath leaving only the sheath in the carotid held in by Lig-a-Loops.

Heparin sodium (Elkins-Sinn) was then administered intravenous (IV) at a dose of 150 units/kg of body weight, with repeated doses of 100 units/kg given every 30 minutes for the duration of the procedure. A Gould pressure transducer was attached to the flush port of the catheter sheath for measurement of MAP. At this time the animal was also started on an IV NTG drip (.25 mg/ml, ICI Americas) at a rate sufficient to drop MAP 5 to 10 mm Hg, a clinically-derived value found to prevent vascular spasm during coronary angiography.

Prior to denudation, a 5 Fr Intravascular Ultrasound Imaging Catheter (Cardiovascular Imaging Systems) was introduced through the catheter sheath and advanced along a guidewire under fluoroscopy into the area of the LADCA selected for later denudation. Cross-sectional images of the arterial lumen generated by the catheter were displayed on a television monitor by the system's base unit (CVIS Insight System, Cardiovascular Imaging Systems, Inc.); concurrent image recording was accomplished by an integral VHS videocassette recorder. Recordings were made before and during injection of 10 and 20 μg ACh bolus doses through a flush port on the catheter. The LADCA was then denuded of endothelium in the manner described next.

The catheter sheath used has a valved opening through which a guide catheter (8 Fr, 100 cm Marathon® Guiding Catheter, Baxter Healthcare Corporation) was advanced under fluoroscopy to the ostium of the LADCA. A steerable guide wire (Hi-Per™ Flex™ Steerable Guide Wire, USCI Division, C.R. Bard) was advanced angiographically through the guide catheter into the LADCA by using radioopaque contrast media (Renografin®-76, Squibb Diagnostics). A balloon wedge pressure catheter (4 Fr, 60 or 110 cm Arrow® Balloon Wedge Pressure Catheter, Arrow International) was advanced over this guide wire and angiographically positioned in the LADCA in an area free of branch vessels. The balloon was then inflated with air until contact was made with the walls of the LADCA, the catheter withdrawn 1 to 2 cm, the balloon deflated, and the catheter readvanced to its previous position. This procedure repeated five times has been shown by others to adequately denude the vessel of normal endothelium for a period of several weeks[93], histopathologic examination of denuded CA segments from the animals in this study confirmed removal of endothelial cells without evidence of regrowth.

Immediately following the denudation the vessel was retested with ACh and ultrasonic images of the lumen were again recorded. All angiographic equipment was then removed, the NTG infusion stopped and the opening in the carotid sutured closed with 5-0 Prolene® monofilament polypropylene suture (Ethicon, Inc.). The neck incision was apposed using 3-0 Ethilon® monofilament nylon (Ethicon, Inc.) in the deeper tissues and the skin closed with Royal®-35W

disposable skin staples (United States Surgical Corporation). Post-surgically the animal received 250 mg IM and 250 mg IV of cefazolin sodium (Zolicef™, Bristol Laboratories) as antibiotic therapy. Animals were reevaluated (as above) 1,3,7, 10 or 30 days post-denudation. After euthanization by an overdose of anesthetic agent, the altered CA was excised and examined by histopathology to verify removal of endothelial cells.

The CVIS Insight System includes an image analysis function that was used on all recorded images. An image was recalled from the videocassette and two operator-controlled cursors were placed maximally apart on opposite sides of the vessel lumen. The distance between the cursors in millimeters was then calculated by the CVIS system and displayed on screen.

Surgical Preparation for Experimental Studies

Healthy domestic Yorkshire/Hampshire cross female or castrated male swine were sedated as above, weighed, and anesthetized with a 3%/33% solution of α-chloralose and urethane (both, Sigma Chemical Company) through a marginal ear vein catheter until a surgical plane of anesthesia/analgesia was attained. After endotracheal intubation, the animal was placed in dorsal recumbency on a V-tray.

The right femoral artery and vein were dissected and isolated. The artery received a 7 Fr Mikro-tip® dual pressure transducer catheter (Millar Instruments) advanced under fluoroscopy across the left atrioventricular valve for simultaneous

measurement of arterial and intraventricular pressures. The vein received an appropriately sized polyethylene catheter for fluid and anesthetic administration. These catheters were sutured into position for the duration of the experiment.

The left external jugular vein and carotid artery were also isolated. The vein received a Sones B 7.5 Fr, 100cm (USCI Division, C.R. Bard) cardiovascular catheter that was advanced under fluoroscopy into the coronary sinus. The left carotid artery received the previously described catheter sheath system as an entry point for CA catheters. The carotid was isolated and retracted with 2-0 silk braided stay sutures (Ethicon) and a small incision made across the wall. The fully assembled catheter sheath system was inserted into the lumen of the vessel, the sheath sutured into position using the stay sutures, and the dilator and wire removed. NTG and heparin were then given as described above. The steerable guide wire was inserted in a similar fashion, but all experimental animals received an infusion catheter (2.5 Fr, 125 cm Infusion Catheter, Cordis Corporation) advanced along this guide wire; the guide wire was withdrawn following placement of the infusion catheter. NTG was stopped at this point and the animal was moved from Radiology to the laboratory for further instrumentation.

In the laboratory the animal was placed on a fluid-filled heating pad (Model K-20, American Hamilton) on a surgical table, right laterally recumbent, and an IV drip of normal saline at a rate of 3 to 5 ml/kg/hr was started. The endotracheal tube was connected to a mechanical respirator (Dual Phase Control Respirator Pump, Harvard Apparatus) cycling at 8 to 12 ventilations per minute with a volume

of 250 to 300 ml. The dual pressure transducer and ECG leads were connected to a physiologic chart recorder (Model RS 3800, Gould Electronics) and a rectal temperature lead was emplaced. Arterial and venous blood gas samples were drawn into heparinized glass syringes and held on ice until assayed if not tested immediately; respirator adjustments and oxygen supplementation were done as necessary to maintain blood gas values within porcine physiologic limits (pH=7.40 to 7.53, PaO_2= 72 to 93, $PaCO_2$= 35 to 44).

A thoracotomy at the left 4th intercostal space was performed and the opening maintained with a Finochetto rib spreader retractor. Heat cautery (300 watt, Geiger Instrument Company) was used extensively during the surgery to control hemostasis. The lung lobes of the left side were packed caudodorsal with saline soaked sponges and the pericardium opened with an inverse 'T' incision, sparing the phrenic nerve and all significant pericardial vessels, and the left auricular appendage was retracted using 3-0 silk (Ethicon) stay sutures.

The LADCA and the LCFXCA were then carefully dissected free of the epicardium for a sufficient length (approximately 1 cm) to allow placement of appropriately sized circumferential electromagnetic blood flow probes (Carolina Medical Electronics). Lidocaine (Xylocaine® 2%, Astra Pharmaceutical Products, Inc.) was applied topically to the exposed coronary artery during the dissection to limit vasospasm. The opening was then covered with plastic film (Saran Wrap™, Dow Chemical Company) and the preparation allowed to stabilize for twenty minutes prior to any intracoronary injections. NTG and SP infusions were begun

at the appropriate time in the concentrations and rates stated in Section 1. NOLA-blockade studies also received 4 mg/kg of body weight NOLA IV 15 minutes prior to experimental measurements.

Statistical Analysis

Data were analyzed by Analysis of Variance (ANOVA) comparing the effect of an ACh dose to an equivalent dose in the presence of a concurrent infusion of either NTG or SP. Significance was set at the $p<0.05$ level. An analysis of covariance was used to determine heterogeneity of slopes for the best-fit curves; individual regression lines were then calculated for differences and significance reported for the $p<0.05$ level.

RESULTS

Denudation Validation Study

No dose of intracoronary ACh produced any change in vessel wall cross-sectional area immediately before or after denudation, indicating a lack of reactivity by the intact and freshly-injured vessels. Animals reevaluated after recovering from the denudation, however, showed maximal vessel reactivity in response to intracoronary ACh 6 to 10 days post-denudation. A total of 4 animals were tested by the method described during this post-denudation period. Upon administration of intracoronary ACh, these showed a reduction in LADCA cross-sectional area of 59% only in the denuded area — other non-denuded areas were non-reactive. The mean baseline diameter was 14.32 mm, with reduction to a mean of 9.15 mm occurring after a 20 μg ACh bolus. All animals reported here had CA segments examined post-mortem by histopathology and were found to have a minimum of 70% and a maximum of 100% of endothelial cells removed. No evidence of regrowth of endothelial cells was noted. The profound spasm of denuded segments clearly demonstrated the critical role of the endothelium in modulating vessel dynamics and thus flow to the myocardium.

Description of Data Analysis

A stylized LADCA flow trace has been drawn in Figure 2 and labelled to show the origin of data values; representative chart recorder LADCA flow tracings

produced by the 1ml bolus injections of saline and the five different ACh doses used are reproduced in Figure 3. Differences between the pre-ACh stable baseline to point of minimum flow (Trough Delta or TD) and point of maximum flow (Hyperemia Delta or HD) were calculated and an analysis of variance (ANOVA) within and between groups was performed on the values obtained. Calculation of areas between the LAD curve and baseline were also done and designated Trough Area (TA) for flow deficit periods and Hyperemia Area (HA) for flow repayment periods. The resulting values were also subjected to ANOVA testing. Differences of $p<.05$ were considered significant. Finally, best-fit lines were applied to mean values with calculation of slope and Y-intercept data, and significance differences were evaluated by an analysis of covariance to determine heterogeneity of slopes.

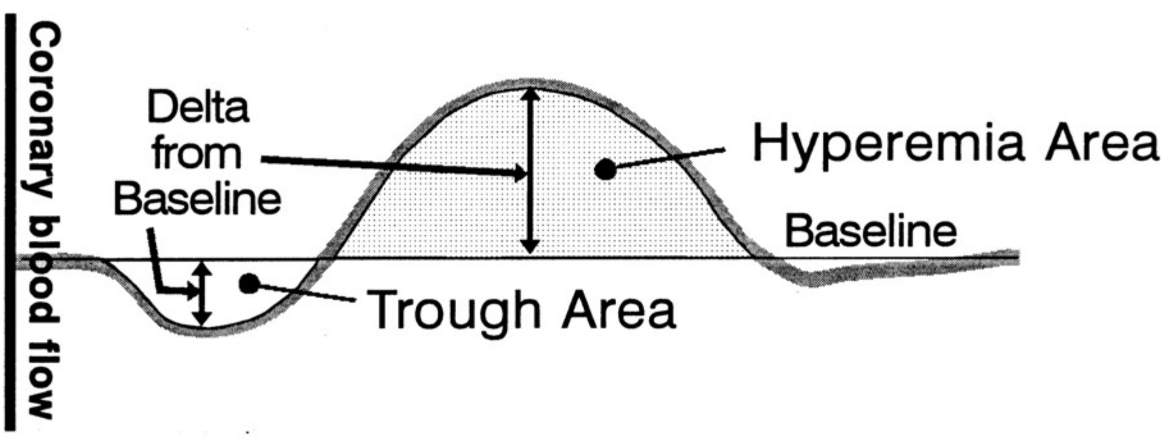

Figure 2. Schematic representation of a stylized LADCA flow trace labelled to show the origin of terms used to describe data values. Striped portions indicate areas while Delta values are the difference between baseline flow and peak or trough.

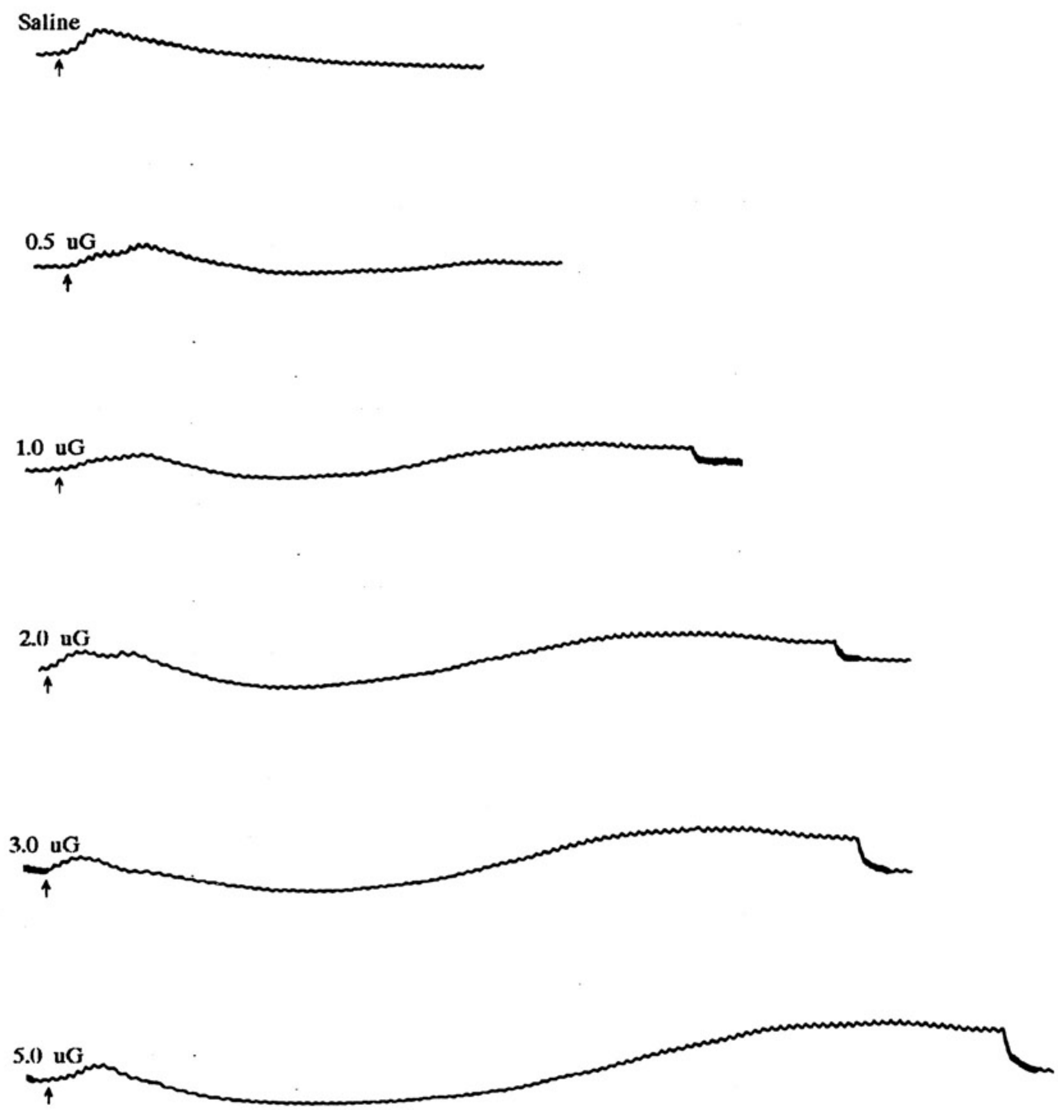

Figure 3. Reproduction of left anterior descending coronary artery flow traces resulting from 1ml intracoronary bolus acetylcholine injections (at arrows). Upward deviation of curve indicates increased flow; the drop at the end of the curve is due to a change in chart recorder speed.

Effect of Dilator Infusions and ACh Injection on Baseline

Figure 4 illustrates mean LADCA flow values (ml/min per 100 grams of tissue perfused) during the hyperemic, baseline and trough periods for each ACh dose injected. Baseline flows were 12.79±1.78 ml/min/100gm before and 13.93±2.05 ml/min/100gm after NTG infusion; for substance P infusion, baseline flows were 12.13±2.34 ml/min/100gm before and 10.00±1.91 ml/min/100gm after initiation.

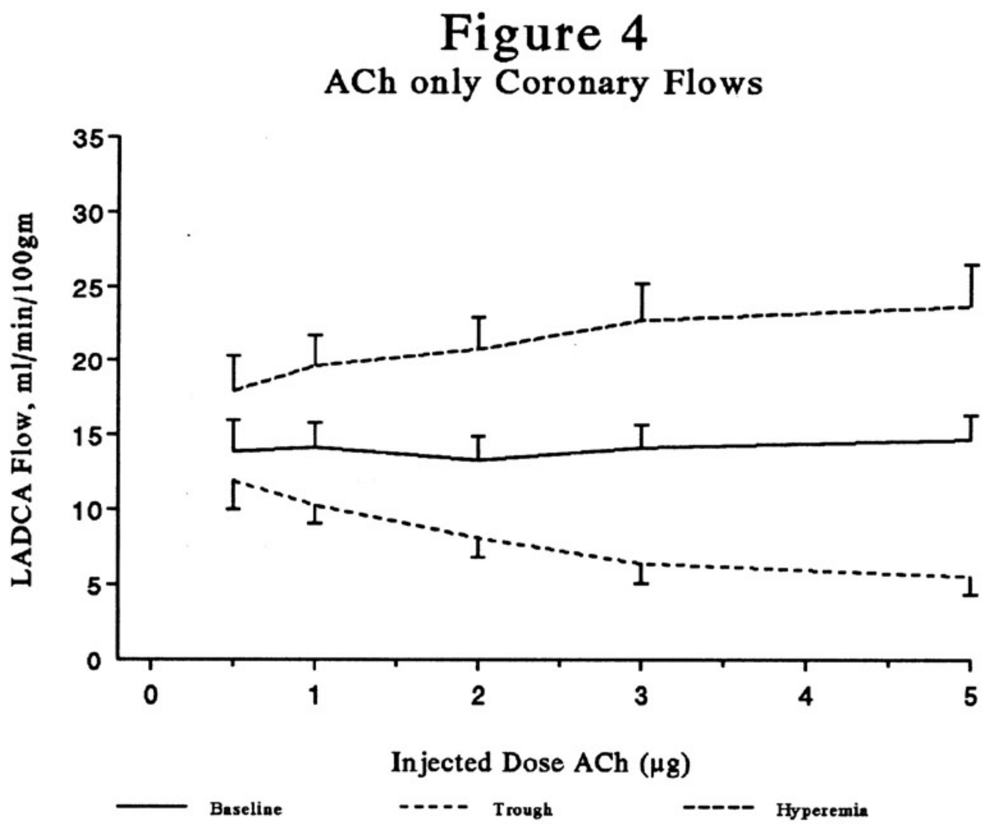

Figure 4. Mean LADCA flow values for hyperemic, baseline and trough periods during ACh bolus dose injection. ACh = acetylcholine; LADCA = left anterior descending coronary artery.

Control Group Study

Mean Delta and Area values for Control Group flow and resistance data are given in Tables 1, 3 and 5. The tables also note any means significantly different from others in the Group. The one notable trend revealed by this method of analysis is the NTG+ACh and SP+ACh difference from ACh only values for the LAD hyperemia delta (LAD HD) which has been graphed in Figure 5. The LAD HD is significantly reduced at the 0.5, 1.0, 2.0 and 3.0 µg doses for the NTG+ACh treated group and at the 0.5 and 1.0 µg doses for the SP+ACh treated group. Other Control Group hemodynamic data consisting of MAP, PIP, HR, PRP, and dP/dt are listed in Tables 2, 4 and 6. Differences (if any) are marked in the Tables for these parameters.

LAD curve Trough and Hyperemia Areas were also evaluated. No additional significance was apparent in the TA data but the HA differences were amplified, particularly at the higher doses of ACh (see HA data in Tables 3 and 5). HA differences were significant ($p<.05$) for the 1.0 through 5.0 µg doses in the NTG+ACh group and at the 2.0 through 5.0 µg doses for the SP+ACh group. This relationship is graphically presented in Figure 6.

Best-fit curves were applied to the means of the TA values; the results are presented as slope and Y-intercept data in Table 7 and graphed in Figure 7. Neither the slopes nor the Y-intercepts of the NTG+ACh and SP+ACh lines are significantly different from the ACh only line at the $p<.05$ level.

TABLE 1
CONTROL GROUP FLOW/RESISTANCE DATA
Delta from baseline

ACh only (no infusion)

ACh dose (μg)		LAD flow ml/min/100gm TD	HD	LAD Area ml/100gm TA	HA	PPCF ml/min TD	HD	CFX flow ml/min TD	HD	CVR mm Hg/ml/min/100g TD	HD
Saline	Mean	-0.65	1.81	-0.14	3.71	-9.27	4.30	0.20	1.40	0.50	-1.99
	SEM ±	0.00	0.13	0.12	1.27	5.09	3.48	0.14	0.64	0.48	0.85
	(n=)	(3)	(3)	(4)	(4)	(3)	(3)	(2)	(2)	(3)	(3)
0.5 uG	Mean	-1.71	4.24	-2.47	4.28	-9.98	17.70	1.42	1.27	0.46	-2.00
	SEM ±	0.26	0.61	0.57	0.97	6.64	3.89	1.22	1.07	0.44	0.64
	(n=)	(7)	(7)	(8)	(8)	(6)	(6)	(6)	(6)	(7)	(7)
1.0 uG	Mean	-3.81	5.80	-4.82	30.94	-22.84	27.86	-0.89	2.91	1.26	-2.01
	SEM ±	0.94	0.84	1.27	9.87	5.11	8.32	0.96	0.64	0.51	0.40
	(n=)	(11)	(11)	(12)	(12)	(10)	(10)	(8)	(8)	(11)	(11)
2.0 uG	Mean	-5.98	7.27	-9.28	38.24	-36.99	44.87	-0.17	3.68	4.39	-2.05
	SEM ±	0.82	1.11	1.22	7.35	6.13	9.18	0.83	0.83	1.56	0.31
	(n=)	(11)	(11)	(12)	(12)	(10)	(10)	(8)	(8)	(11)	(11)
3.0 uG	Mean	-7.73	9.44	-15.80	59.57	-53.89	44.71	-0.14	4.39	17.62	-2.29
	SEM ±	1.05	1.68	2.57	12.76	10.21	5.98	1.14	1.10	8.33	0.32
	(n=)	(11)	(11)	(12)	(12)	(10)	(10)	(8)	(8)	(11)	(11)
5.0 uG	Mean	-9.26	9.87	-24.58	113.65	-59.58	46.37	-6.27	7.31	188.77	-2.19
	SEM ±	1.22	2.15	4.10	34.07	9.90	8.38	3.01	1.78	168.70	0.34
	(n=)	(10)	(10)	(11)	(11)	(9)	(9)	(7)	(7)	(10)	(10)

LAD = Left Anterior Descending Coronary Artery
LAD Area = Volume above/below baseline
PPCF = Peak Phasic Coronary Flow
CFX = Circumflex Coronary Artery
CVR = Coronary Vascular Resistance

TD = Trough Delta
HD = Hyperemia Delta
TA = Trough Area
HA = Hyperemia Area
ACh = Acetylcholine

TABLE 2
CONTROL GROUP HEMODYNAMIC DATA
Delta from baseline

ACh only (no infusion)

ACh dose (μg)		MAP mm Hg TD	MAP mm Hg HD	PIP mm Hg TD	PIP mm Hg HD	HR beats/min TD	HR beats/min HD	PRP mm Hg/min TD	PRP mm Hg/min HD	dP/dT mm Hg/min TD	dP/dT mm Hg/min HD
Saline	Mean	-4.33	-0.33	-4.00	-0.33	-4.67	-5.67	-1335.33	-696.00	17.33	144.33
	SEM ±	1.52	0.72	1.63	0.98	6.77	5.86	613.02	808.21	106.30	53.72
	(n=)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
0.5 uG	Mean	-6.29	-1.43	-6.57	-1.71	1.57	-0.14	-892.43	-226.86	-376.43	-176.00
	SEM ±	3.14	1.31	2.89	1.16	1.37	0.37	500.73	186.77	49.76	33.78
	(n=)	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(7)
1.0 uG	Mean	-10.36	-0.73	-10.18	-0.64	3.09	5.27	-1284.09	518.36	-467.45	-73.64
	SEM ±	2.04	1.47	1.88	1.39	1.03	1.42	302.77	231.75	77.04	95.06
	(n=)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)
2.0 uG	Mean	-11.82	1.91	-11.09	1.82	0.73	2.18	-1736.55	485.64	-726.36	-40.09
	SEM ±	2.18	1.00	2.03	0.82	2.10	1.03	390.18	121.82	73.60	83.89
	(n=)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)
3.0 uG	Mean	-12.36	1.27	-12.18	1.18	0.91	1.45	-1805.09	325.82	-796.73	-33.91
	SEM ±	1.93	1.23	1.68	1.02	0.89	1.15	307.21	234.19	90.61	88.38
	(n=)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)
5.0 uG	Mean	-19.90	0.40	-18.40	0.30	2.40	3.60	-2597.30	301.90	-972.80	-149.70
	SEM ±	1.84	1.56	1.68	1.37	1.42	2.37	303.82	242.37	92.15	72.60
	(n=)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)

MAP = Mean Arterial Pressure
PIP = Peak Intraventricular Pressure
HR = Heart Rate
PRP = Pressure Rate Product
dP/dt = Change in Pressure per Time

TD = Trough Delta
HD = Hyperemia Delta
ACh = Acetylcholine

TABLE 3
CONTROL GROUP FLOW/RESISTANCE DATA
Delta from baseline

Nitroglycerin infused (NTG + ACh)
* = Different from ACh only at p<.05

ACh dose (μg)		LAD flow ml/min/100gm TD	LAD flow ml/min/100gm HD	LAD Area ml/100gm TA	LAD Area ml/100gm HA	PPCF ml/min TD	PPCF ml/min HD	CFX flow ml/min TD	CFX flow ml/min HD	CVR mmHg/ml/min/100g TD	CVR mmHg/ml/min/100g HD
Saline	Mean	-0.54	0.66	0.00	1.74	-4.60	5.00	-1.10	0.40	-0.89	-0.47
	SEM ±	--	--	--	--	--	--	--	--	--	--
	(n=)	(1)	(1)	(2)	(2)	(1)	(1)	(1)	(1)	(1)	(1)
0.5 uG	Mean	-0.93 *	2.99 *	-0.49	2.01	2.28 *	21.67	0.22	0.87	0.07	-1.21
	SEM ±	0.53	0.59	0.19	1.33	2.48	3.22	0.75	0.53	0.26	0.43
	(n=)	(7)	(7)	(8)	(7)	(6)	(6)	(6)	(6)	(7)	(7)
1.0 uG	Mean	-3.18	3.04 *	-4.89	9.16 *	-19.02	23.42 *	0.54	2.33	1.85	-1.02 *
	SEM ±	1.00	0.32	1.75	2.08	6.49	4.96	0.75	1.21	0.99	0.35
	(n=)	(10)	(10)	(11)	(11)	(9)	(9)	(8)	(8)	(10)	(10)
2.0 uG	Mean	-4.29	2.81 *	-8.06	12.45 *	-19.82 *	17.91	-0.36	1.84	3.36	-1.14
	SEM ±	1.24	0.56	2.04	2.46	2.97	4.88	0.69	1.15	1.66	0.43
	(n=)	(10)	(10)	(11)	(11)	(9)	(9)	(8)	(8)	(10)	(10)
3.0 uG	Mean	-6.79	3.26 *	-13.28	14.54 *	-25.60	25.21	-1.19	1.90	6.74	-1.14 *
	SEM ±	1.37	0.86	2.82	3.63	7.10	5.26	0.76	1.39	4.12	0.39
	(n=)	(10)	(10)	(11)	(11)	(9)	(9)	(8)	(8)	(10)	(10)
5.0 uG	Mean	-8.63	5.09	-18.02	28.09 *	-31.10 *	34.24	-2.57	3.06 *	323.68	-1.36
	SEM ±	1.38	1.27	3.06	5.61	6.00	8.65	1.01	1.41	302.42	0.45
	(n=)	(10)	(10)	(11)	(11)	(9)	(9)	(8)	(8)	(10)	(10)

LAD = Left Anterior Descending Coronary Artery
LAD Area = Volume above/below baseline
PPCF = Peak Phasic Coronary Flow
CFX = Circumflex Coronary Artery
CVR = Coronary Vascular Resistance

ACh = Acetylcholine
TD = Trough Delta
HD = Hyperemia Delta
TA = Trough Area
HA = Hyperemia Area

TABLE 4
CONTROL GROUP HEMODYNAMIC DATA
Delta from baseline

Nitroglycerin infused (NTG+ACh)

ACh dose (μg)		MAP mm Hg TD	MAP mm Hg HD	PIP mm Hg TD	PIP mm Hg HD	HR beats/min TD	HR beats/min HD	PRP mm Hg/min TD	PRP mm Hg/min HD	dP/dT mm Hg/min TD	dP/dT mm Hg/min HD
Saline	Mean	-12.00	0.00	-11.00	1.00	-2.00	-2.00	-2259.00	33.00	-357.00	49.00
	SEM ±	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(n=)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
0.5 uG	Mean	-3.57	-1.00	-1.00	-2.86	2.43	0.29	196.14	-461.14	-248.29	-24.86 *
	SEM ±	2.29	0.40	3.74	2.47	2.10	0.87	982.47	577.04	82.18	66.23
	(n=)	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(7)
1.0 uG	Mean	-6.00	2.00	-9.10	-2.20	-0.10	0.00	-1595.00	-389.80	-320.60	-69.40
	SEM ±	1.98	1.42	2.68	3.61	0.46	0.51	532.53	689.18	80.23	80.15
	(n=)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
2.0 uG	Mean	-7.60	1.60	-11.40	-2.60	-0.10	0.50	-2048.40	-409.90	-552.50	-58.90
	SEM ±	2.65	1.76	3.55	3.98	0.64	0.86	736.79	762.93	113.52	68.69
	(n=)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
3.0 uG	Mean	-10.60	0.60	-13.20	0.90	-0.30	-0.80	-2285.80	114.80	-540.50	-27.80
	SEM ±	2.72	1.78	3.24	1.72	0.58	0.85	745.42	292.51	125.11	56.72
	(n=)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
5.0 uG	Mean	-12.20 *	2.60	-15.30	-2.10	-1.10 *	-0.70	-2631.20	-563.10	-659.70	-163.20
	SEM ±	2.43	2.03	3.21	4.72	0.52	1.52	695.87	869.31	110.72	65.27
	(n=)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)

MAP = Mean Arterial Pressure
PIP = Peak Intraventricular Pressure
HR = Heart Rate
PRP = Pressure Rate Product
dP/dt = Change in Pressure per Time

* = Different from ACh only at p<.05

ACh = Acetylcholine
TD = Trough Delta
HD = Hyperemia Delta

TABLE 5
CONTROL GROUP FLOW/RESISTANCE DATA
Delta from baseline

Substance P infused (SP+ACh)
* = Different from ACh only at p<.05

ACh dose (µg)		LAD flow ml/min/100gm TD	LAD flow HD	LAD Area ml/100gm TA	LAD Area HA	PPCF ml/min TD	PPCF HD	CFX flow ml/min TD	CFX flow HD	CVR mm Hg/ml/min/100g TD	CVR HD
Saline	Mean	-0.99	1.11	-0.61	2.13	0.30	13.90	-1.50	1.20	-0.41	-0.39
	SEM ±	0.00	0.00	0.43	0.92	0.00	0.00	0.00	0.00	0.00	0.00
	(n=)	(1)	(1)	(2)	(2)	(1)	(1)	(1)	(1)	(1)	(1)
0.5 uG	Mean	-1.03 *	1.85 *	-0.55	3.19	-4.90	-0.40	-2.40	2.20	0.04	-0.18
	SEM ±	0.19	0.12	0.45	0.87	1.27	5.87	0.00	0.00	0.37	0.02
	(n=)	(2)	(2)	(3)	(3)	(2)	(2)	(1)	(1)	(2)	(2)
1.0 uG	Mean	-4.39	2.99 *	-7.65	16.31	-15.78	8.24 *	-1.00	1.95	3.29	-0.97 *
	SEM ±	0.95	0.92	3.45	6.14	4.46	4.59	0.71	0.11	1.36	0.38
	(n=)	(5)	(5)	(6)	(6)	(5)	(5)	(2)	(2)	(5)	(5)
2.0 uG	Mean	-5.90	5.06	-10.17	18.19 *	-35.68	24.72	-2.75	5.95	6.21	-1.07
	SEM ±	0.99	2.18	3.55	4.74	8.74	11.91	0.25	3.64	3.10	0.51
	(n=)	(5)	(5)	(6)	(6)	(5)	(5)	(2)	(2)	(5)	(5)
3.0 uG	Mean	-6.65	5.45	-9.86	23.19 *	-35.08	28.16	-3.60	3.55	320.41	-1.14
	SEM ±	1.58	2.69	3.62	6.23	14.57	11.71	0.35	2.16	284.05	0.57
	(n=)	(5)	(5)	(6)	(6)	(5)	(5)	(2)	(2)	(5)	(5)
5.0 uG	Mean	-8.00	7.89	-16.68	37.86 *	-34.78	29.92	-2.85	1.40 *	363.81	-1.58
	SEM ±	2.06	2.05	5.66	12.51	18.30	10.86	0.11	0.99	318.51	0.32
	(n=)	(5)	(5)	(6)	(6)	(5)	(5)	(2)	(2)	(5)	(5)

LAD = Left Anterior Descending Coronary Artery
LAD Area = Volume above/below baseline
PPCF = Peak Phasic Coronary Flow
CFX = Circumflex Coronary Artery
CVR = Coronary Vascular Resistance

ACh = Acetylcholine
TD = Trough Delta
HD = Hyperemia Delta
TA = Trough Area
HA = Hyperemia Area

TABLE 6
CONTROL GROUP HEMODYNAMIC DATA
Delta from baseline

Substance P infused (SP+ACh)

ACh dose (µg)		MAP mm Hg TD	MAP mm Hg HD	PIP mm Hg TD	PIP mm Hg HD	HR beats/min TD	HR beats/min HD	PRP mm Hg/min TD	PRP mm Hg/min HD	dP/dT mm Hg/min TD	dP/dT mm Hg/min HD
Saline	Mean	-9.00	0.00	-10.00	1.00	2.00	1.00	-1774.00	264.00	-402.00	127.00
	SEM ±	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(n=)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
0.5 uG	Mean	-4.00	5.50 *	-4.00	5.00	-1.00	-1.00	-856.00	863.00	-188.00	187.00 *
	SEM ±	4.95	0.35	4.24	2.12	1.41	1.41	692.96	522.55	222.74	185.97
	(n=)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
1.0 uG	Mean	-6.80	2.00	-10.60	-2.20	-15.20 *	-15.20	-3726.80	-2212.40	-214.40	51.60
	SEM ±	2.32	1.13	2.38	2.93	13.87	15.62	1994.47	2288.97	261.59	187.52
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
2.0 uG	Mean	-6.60	5.40	-10.00	3.60	-9.40	-6.80	-2751.00	-134.60	-413.00	247.20 *
	SEM ±	2.18	1.82	1.20	2.03	7.75	8.12	910.36	1149.64	217.30	81.22
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
3.0 uG	Mean	-9.20	3.00	-12.20	0.40	-24.20	-24.60	-4372.20	-2195.20	-493.00	29.00
	SEM ±	1.25	1.60	1.28	3.18	22.77	23.00	2268.48	2607.91	234.56	150.77
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
5.0 uG	Mean	-10.20 *	2.60	-10.60	3.60	-2.20 *	-1.00	-2010.80	504.00	-795.20	62.00
	SEM ±	1.82	1.15	1.46	1.34	2.11	3.01	331.42	393.55	129.86	106.73
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)

MAP = Mean Arterial Pressure
PIP = Peak Intraventricular Pressure
HR = Heart Rate
PRP = Pressure Rate Product
dP/dt = Change in Pressure per Time

* = Different from ACh only at $p<.05$

ACh = Acetylcholine
TD = Trough Delta
HD = Hyperemia Delta

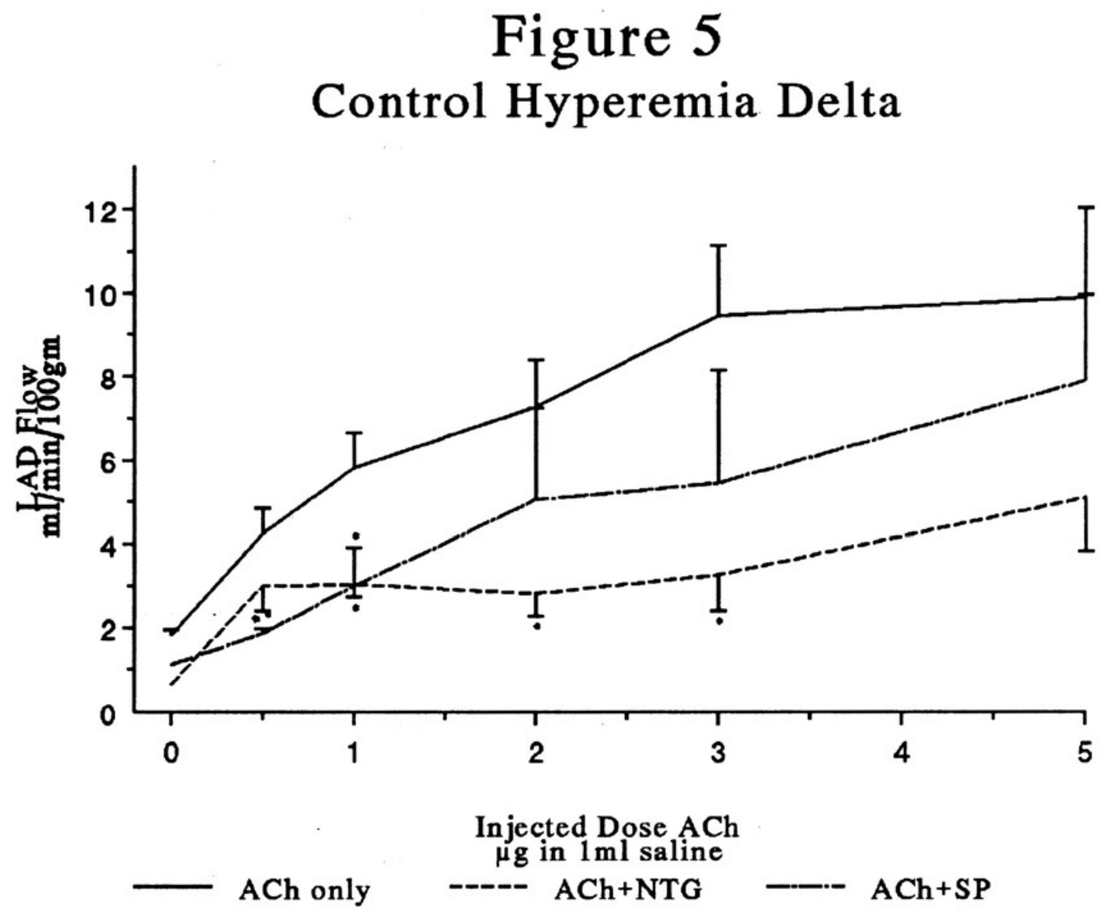

Figure 5. Control Hyperemia Delta, mean values ± SEM. LAD = left anterior descending coronary artery, ACh = acetylcholine, NTG = nitroglycerin, SP = substance P. * = p < .05.

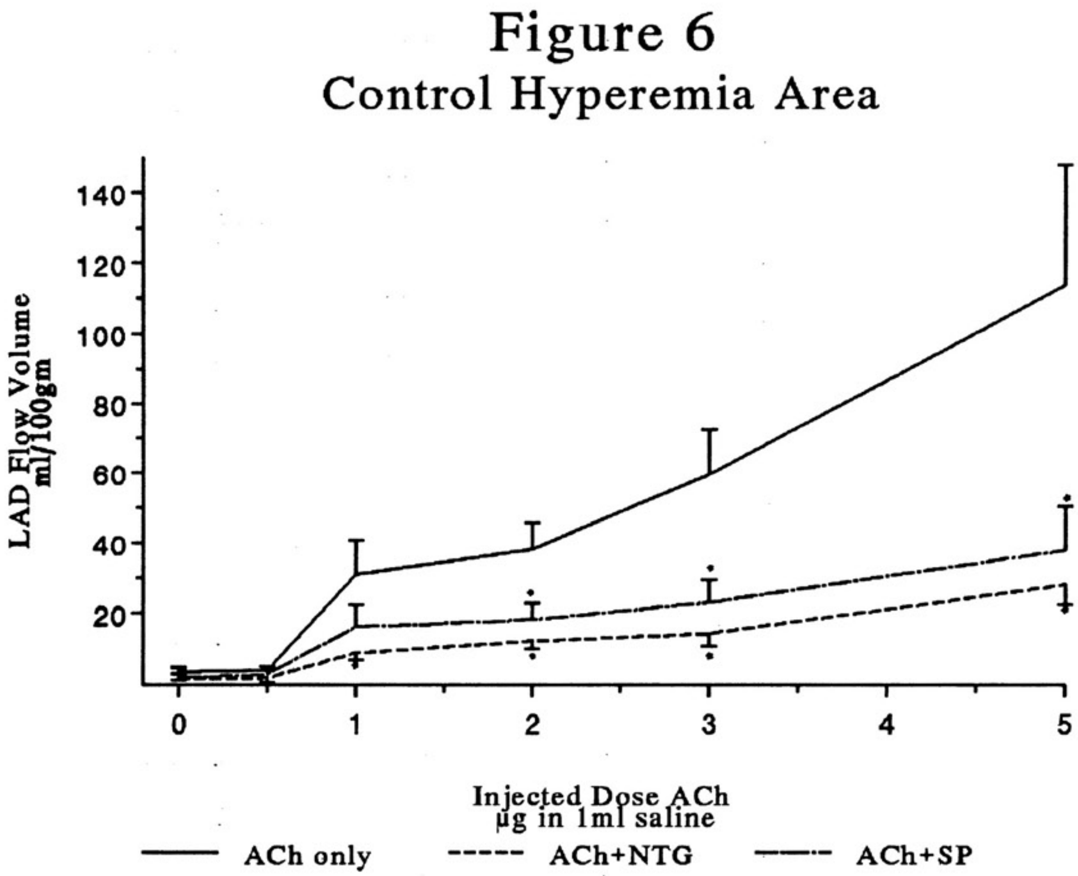

Figure 6. Control Hyperemia Area, mean values ± SEM. LAD = left anterior descending coronary artery, ACh = acetylcholine, NTG = nitroglycerin, SP = substance P. * = p < .05.

TABLE 7

CONTROL TROUGH AREA
Slope and Y-intercept Data

Treatment	Slope	R-value	Y-intercept
ACh only	-5.22	0.998	0.11
NTG+ACh	-4.15	0.985	-0.04
SP+ACh	-3.87	0.968	-1.57

ACh only = ACh Dose Response Curve
NTG+ACh = ACh DRC during NTG infusion
SP+ACh = ACh DRC during SP infusion

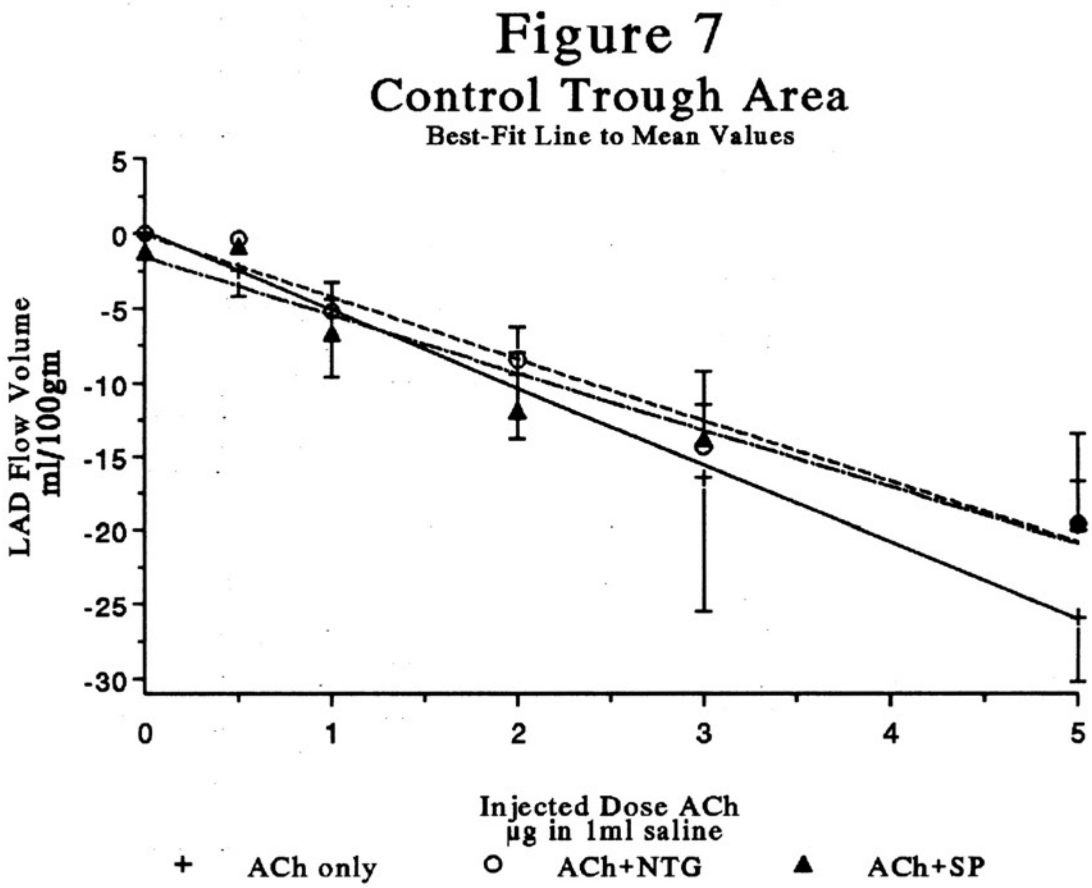

Figure 7. Control Trough Area, best-fit line to mean values ± SEM. LAD = left anterior descending coronary artery, ACh = acetylcholine, NTG = nitroglycerin, SP = substance P.

NOLA-blocked Group Study

Tables 8, 10 and 12 contain Delta and Area means and significant difference annotations for the NOLA-blocked Group flow and resistance data. Tables 9, 11 and 13 list the additional hemodynamic data and any significance. No significant difference from the ACh only group was noted for the Trough Delta or Area data. The LAD HD is graphed in Figure 8; significant differences ($p<.05$) exist for the NTG+ACh group at the 3.0 μg dose and for the SP+ACh group at the 0.5 and 1.0 μg doses. No differences were noted for the TA data set either, but HA differences were significant (from the ACh only group) for the SP+ACh group at the 2.0, 3.0 and 5.0 μg doses.

Regression analysis of the NOLA TA and HA values are presented as slope and Y-intercept data in Table 14 and graphed in Figures 9 and 10, respectively. No differences were noted in either data set for Y-intercept values. In both data sets the slope of the NTG+ACh and SP+ACh lines differed significantly ($p<.05$) from the ACh only slope but were not significantly different from each other.

TABLE 8
NOLA-BLOCKED GROUP FLOW/RESISTANCE DATA
Delta from baseline

ACh only (no infusion)

ACh dose (μg)		LAD flow ml/min/100gm TD	LAD flow HD	LAD Area ml/100gm TA	LAD Area HA	PPCF ml/min TD	PPCF HD	CFX flow ml/min TD	CFX flow HD	CVR mm Hg/ml/min/100g TD	CVR HD
Saline	Mean	-2.49	3.07	-0.03	2.52	0.46	16.80	0.38	-0.28	0.58	-0.75
	SEM ±	0.56	0.66	0.02	0.76	3.98	3.08	0.49	0.25	0.29	0.37
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(4)	(4)	(5)	(5)
0.5 uG	Mean	-3.45	3.77	-2.39	12.89	-5.38	17.08	-0.27	0.20	-0.10	-1.15
	SEM ±	1.25	0.58	1.00	8.87	6.90	2.70	0.67	0.43	0.47	0.47
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(5)	(5)
1.0 uG	Mean	-8.71	9.19	-7.94	17.70	-44.56	30.52	-0.32	3.63	2.18	-1.78
	SEM ±	2.84	3.33	2.10	5.42	12.18	8.16	1.00	0.72	1.25	0.64
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(4)	(4)	(5)	(5)
2.0 uG	Mean	-11.66	17.75	-14.44	61.74	-56.22	55.28	-2.42	3.50	5.17	-2.63
	SEM ±	3.85	5.11	3.85	16.17	13.99	10.00	1.37	0.79	2.78	0.95
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(4)	(4)	(5)	(5)
3.0 uG	Mean	-11.91	23.23	-17.93	73.26	-58.04	70.24	-5.23	7.95	6.39	-3.07
	SEM ±	3.81	5.82	3.69	14.85	11.68	8.31	2.96	1.24	3.59	1.00
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(4)	(4)	(5)	(5)
5.0 uG	Mean	-15.23	27.99	-31.12	195.24	-65.53	85.10	0.85	3.95	2.63	-2.79
	SEM ±	4.22	5.84	14.06	92.79	11.87	14.00	3.36	4.91	0.84	1.21
	(n=)	(4)	(4)	(4)	(4)	(4)	(4)	(2)	(2)	(4)	(4)

LAD = Left Anterior Descending Coronary Artery
LAD Area = Volume above/below baseline
PPCF = Peak Phasic Coronary Flow
CFX = Circumflex Coronary Artery
CVR = Coronary Vascular Resistance

ACh = Acetylcholine
TD = Trough Delta
HD = Hyperemia Delta
TA = Trough Area
HA = Hyperemia Area

TABLE 9
NOLA-BLOCKED GROUP HEMODYNAMIC DATA
Delta from baseline

ACh only (no infusion)

ACh dose (μg)		MAP mm Hg		PIP mm Hg		HR beats/min		PRP mm Hg/min		dP/dT mm Hg/min	
		TD	HD	TD	HD	TD	HD	TD	HD	TD	HD
Saline	Mean	-0.40	-0.20	-0.40	-0.60	1.80	1.40	170.40	73.60	-156.00	-143.20
	SEM ±	0.83	0.95	0.83	0.83	0.33	0.46	158.84	137.62	94.25	69.66
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
0.5 uG	Mean	-6.40	-0.60	-5.60	-0.80	-3.60	2.60	-1138.00	156.60	-178.80	-22.00
	SEM ±	4.41	1.43	4.35	1.43	5.77	2.05	776.50	346.78	52.63	75.52
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
1.0 uG	Mean	-10.00	-6.80	-10.00	-6.20	1.60	2.60	-1270.80	-640.60	-479.20	-148.40
	SEM ±	2.06	4.93	2.06	4.80	0.36	1.78	261.45	577.75	49.71	108.34
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
2.0 uG	Mean	-13.00	-3.20	-13.20	-2.40	1.40	5.00	-1727.00	249.80	-528.80	35.00
	SEM ±	2.06	4.74	1.97	4.80	1.43	1.74	372.33	628.58	96.10	132.89
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
3.0 uG	Mean	-19.80	-2.80	-19.40	-1.80	2.20	6.60	-2633.20	530.80	-574.60	137.60
	SEM ±	3.65	3.05	3.79	3.41	1.04	1.40	529.86	507.67	84.19	126.99
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
5.0 uG	Mean	-26.25	-1.00	-25.50	-0.25	4.50	5.50	-3356.50	537.75	-680.50	55.25
	SEM ±	2.33	4.11	2.80	4.01	1.03	3.72	373.14	411.94	126.47	204.38
	(n=)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)

MAP = Mean Arterial Pressure
PIP = Peak Intraventricular Pressure
HR = Heart Rate
PRP = Pressure Rate Product
dP/dt = Change in Pressure per Time

TD = Trough Delta
HD = Hyperemia Delta
ACh = Acetylcholine

TABLE 10
NOLA-BLOCKED GROUP FLOW/RESISTANCE DATA
Delta from baseline

Nitroglycerin infused (NTG + ACh)
* = Different from ACh only at $p<.05$

ACh dose (μg)		LAD flow ml/min/100gm TD	LAD flow ml/min/100gm HD	LAD Area ml/100gm TA	LAD Area ml/100gm HA	PPCF ml/min TD	PPCF ml/min HD	CFX flow ml/min TD	CFX flow ml/min HD	CVR mm Hg/ml/min/100g TD	CVR mm Hg/ml/min/100g HD
Saline	Mean	-2.44	5.68	0.00	6.51	13.80	28.88	-0.47	1.83	0.51	-1.19
	SEM ±	0.94	0.88	0.00	4.19	6.13	5.57	0.24	1.29	0.19	0.40
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(5)	(5)
0.5 uG	Mean	-2.98	4.81	-0.09	2.98	-6.50	27.84	-0.87	0.90	-0.26	-1.53
	SEM ±	1.50	0.82	0.08	1.62	8.93	4.97	0.89	0.22	0.46	0.59
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(5)	(5)
1.0 uG	Mean	-5.11	5.43	-2.51	14.97	-16.72	26.78	0.77	3.40	0.60	-1.68
	SEM ±	2.13	1.23	0.81	6.51	8.67	6.19	0.72	2.05	0.46	0.65
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(5)	(5)
2.0 uG	Mean	-8.76	12.12	-8.69	27.71	-41.14	49.16	-0.67	3.57	3.23	-2.57
	SEM ±	2.51	2.68	1.44	5.58	6.57	7.48	2.34	0.53	1.40	0.92
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(5)	(5)
3.0 uG	Mean	-10.33	16.44 *	-12.48	48.68	-46.80	60.52	-1.90	5.30	3.39	-2.94
	SEM ±	2.90	3.62	2.21	9.47	6.15	5.46	2.51	0.93	1.09	0.98
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(5)	(5)
5.0 uG	Mean	-11.02	22.22	-18.66	79.58	-49.54	73.88	2.97	14.40	5.64	-3.00
	SEM ±	2.88	4.41	3.44	13.99	6.59	4.80	3.42	5.78	2.71	0.99
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(5)	(5)

LAD = Left Anterior Descending Coronary Artery
LAD Area = Volume above/below baseline
PPCF = Peak Phasic Coronary Flow
CFX = Circumflex Coronary Artery
CVR = Coronary Vascular Resistance

ACh = Acetylcholine
TD = Trough Delta
HD = Hyperemia Delta
TA = Trough Area
HA = Hyperemia Area

TABLE 11
NOLA-BLOCKED GROUP HEMODYNAMIC DATA
Delta from baseline

Nitroglycerin infused (NTG+ACh)

ACh dose (μg)		MAP mm Hg		PIP mm Hg		HR beats/min		PRP mm Hg/min		dP/dT mm Hg/min	
		TD	HD	TD	HD	TD	HD	TD	HD	TD	HD
Saline	Mean	-1.80	-0.60	-2.00	-0.60	2.60	2.40	-14.00	194.40	-25.80	24.20
	SEM ±	0.91	1.04	1.02	1.22	1.22	0.46	225.47	155.35	42.26	118.71
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
0.5 uG	Mean	-8.60	-2.80	-7.80	-2.60	3.00	2.40	-759.40	-77.60	-94.00	2.40
	SEM ±	4.39	1.73	4.14	1.76	1.67	0.83	383.49	156.09	86.99	170.58
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
1.0 uG	Mean	-9.00	-5.80	-8.20	-4.80	1.40	1.40	-945.00	-525.80	-271.00	21.60
	SEM ±	4.64	4.41	4.67	4.19	0.67	1.37	559.74	408.38	60.48	139.20
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
2.0 uG	Mean	-12.00	-4.80	-11.80	-4.20	2.20	4.00	-1382.20	-94.00	-419.80	44.00
	SEM ±	3.76	5.03	3.77	4.96	0.52	0.63	448.23	633.42	79.27	107.56
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
3.0 uG	Mean	-18.00	-4.00	-17.60	-3.20	1.20	1.40	-2375.00	-398.00	-527.40	52.60
	SEM ±	3.85	3.29	3.79	3.55	2.22	2.59	548.67	362.72	72.53	108.81
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
5.0 uG	Mean	-15.80	0.80	-15.00	2.60	2.40	3.20	-1827.40	706.20	-530.00	287.00
	SEM ±	2.32	2.14	2.15	2.43	1.08	2.20	216.64	138.47	120.52	132.84
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)

MAP = Mean Arterial Pressure
PIP = Peak Intraventricular Pressure
HR = Heart Rate
PRP = Pressure Rate Product
dP/dt = Change in Pressure per Time

ACh = Acetylcholine
TD = Trough Delta
HD = Hyperemia Delta

TABLE 12
NOLA-BLOCKED GROUP FLOW/RESISTANCE DATA
Delta from baseline

Substance P infused (SP+ACh)
* = Different from ACh only at $p<.05$

ACh dose (μg)		LAD flow ml/min/100gm TD	LAD flow ml/min/100gm HD	LAD Area ml/100gm TA	LAD Area ml/100gm HA	PPCF ml/min TD	PPCF ml/min HD	CFX flow ml/min TD	CFX flow ml/min HD	CVR mm Hg/ml/min/100g TD	CVR mm Hg/ml/min/100g HD
Saline	Mean	-1.99	3.93	-0.03	1.41	1.14	23.28	-0.47	6.23 *	0.27	-1.20
	SEM ±	2.14	1.92	0.03	1.06	6.96	8.67	0.38	1.76	0.51	0.46
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(5)	(5)
0.5 uG	Mean	-3.95	1.49 *	-1.83	3.64	-8.44	7.42	-2.03	-0.13	1.94	-0.65
	SEM ±	1.30	0.56	0.87	2.44	9.82	3.46	2.52	0.33	1.09	0.47
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(5)	(5)
1.0 uG	Mean	-7.71	1.31 *	-2.65	7.47	-22.12	6.98	1.40	2.13	2.11	-1.41
	SEM ±	3.87	2.43	0.58	1.78	16.72	10.96	2.96	2.79	0.95	0.68
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(5)	(5)
2.0 uG	Mean	-8.55	8.67	-9.32	22.84 *	-30.80	32.50	-1.97	4.03	9.88	-2.01
	SEM ±	2.45	1.79	1.96	3.52	10.92	6.64	1.53	1.48	6.94	0.85
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(5)	(5)
3.0 uG	Mean	-10.07	13.05	-10.90	41.65 *	-41.94	49.34	-2.13	3.00 *	5.03	-2.25
	SEM ±	4.21	3.12	3.43	12.21	10.27	10.65	1.10	0.92	2.31	0.94
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(5)	(5)
5.0 uG	Mean	-12.23	18.94	-16.99	61.67 *	-50.32	64.76	-4.57	3.77	269.97	-2.56
	SEM ±	4.59	5.54	4.86	14.17	11.90	15.07	1.49	2.34	237.69	0.97
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(5)	(5)

LAD = Left Anterior Descending Coronary Artery
LAD Area = Volume above/below baseline
PPCF = Peak Phasic Coronary Flow
CFX = Circumflex Coronary Artery
CVR = Coronary Vascular Resistance

ACh = Acetylcholine
TD = Trough Delta
HD = Hyperemia Delta
TA = Trough Area
HA = Hyperemia Area

TABLE 13
NOLA-BLOCKED GROUP HEMODYNAMIC DATA
Delta from baseline

Substance P infused (SP+ACh)

ACh dose (μg)		MAP mm Hg		PIP mm Hg		HR beats/min		PRP mm Hg/min		dP/dt mm Hg/min	
		TD	HD	TD	HD	TD	HD	TD	HD	TD	HD
Saline	Mean	-10.00 *	1.20	-9.40	1.20	3.60	2.40	-1015.80	476.40	-184.40	-50.20
	SEM ±	3.06	2.03	3.13	2.07	3.13	3.33	703.57	352.82	59.25	59.14
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
0.5 uG	Mean	-3.60	-6.80 *	-3.20	-6.00	1.40	4.20	-310.80	-449.20	-252.40	-221.60
	SEM ±	1.76	3.22	1.48	2.81	1.91	1.56	221.25	353.64	20.50	79.48
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
1.0 uG	Mean	-9.60	-7.60	-9.40	-7.20	-0.80 *	3.40	-1494.80	-699.20	-320.60	-97.00
	SEM ±	3.55	3.86	3.40	3.55	0.59	1.00	472.29	512.49	66.70	91.83
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
2.0 uG	Mean	-11.80	-2.80	-11.20	-2.40	1.00	7.60	-1462.00	524.80	-417.00	70.60
	SEM ±	3.54	3.41	3.33	3.27	1.20	2.27	398.41	507.80	89.88	170.97
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
3.0 uG	Mean	-14.20	3.80	-12.80	4.80	1.80	4.20	-1539.60	1287.40	-422.00	219.20
	SEM ±	3.99	2.69	3.87	2.63	1.84	4.22	392.14	413.17	93.20	142.98
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
5.0 uG	Mean	-20.60	1.20	-19.00	2.60	1.60	1.80	-2488.80	554.00	-522.00	33.20
	SEM ±	5.02	2.94	4.71	3.22	2.41	1.21	474.83	476.24	131.74	132.12
	(n=)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)

MAP = Mean Arterial Pressure
PIP = Peak Intraventricular Pressure
HR = Heart Rate
PRP = Pressure Rate Product
dP/dt = Change in Pressure per Time

* = Different from ACh only at $p<.05$

ACh = Acetylcholine
TD = Trough Delta
HD = Hyperemia Delta

Figure 8
NOLA Hyperemia Delta

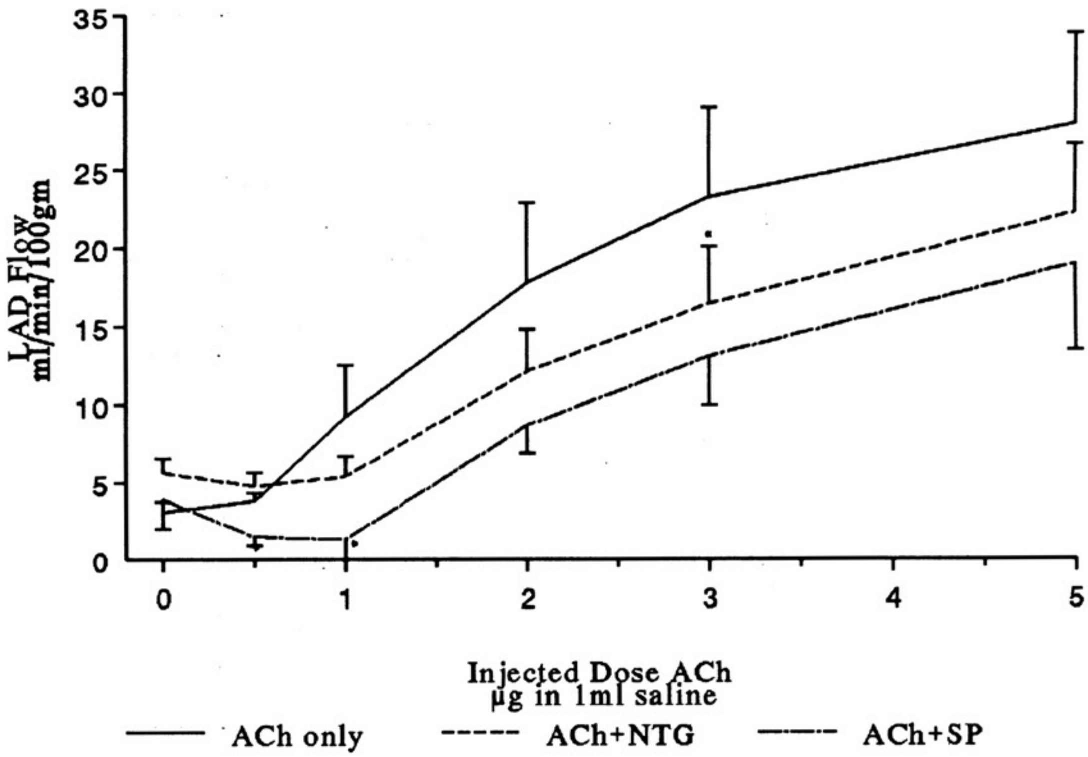

Figure 8. NOLA Hyperemia Delta, mean values ± SEM. NOLA = N^{ω}-nitro-L-arginine, LAD = left anterior descending coronary artery, ACh = acetylcholine, NTG = nitroglycerin, SP = substance P. * = $p < .05$.

TABLE 14

NOLA Experiments
LAD Area Slope and Y-intercept Data

Trough Area

Treatment	Slope	R-value	Y-intercept
ACh only	-6.15	0.994	-0.52
NTG+ACh	-4.03 *	0.989	0.65
SP+ACh	-3.48 *	0.985	-0.28

Hyperemia Area

Treatment	Slope	R-value	Y-intercept
ACh only	37.61	0.971	-11.52
NTG+ACh	15.69 *	0.990	0.0
SP+ACh	12.93 *	0.991	- 1.67

ACh only = ACh Dose Response Curve
NTG+ACh = ACh DRC during Nitroglycerin infusion
SP+ACh = ACh DRC during Substance P infusion

* = different from ACh only at $p<.05$

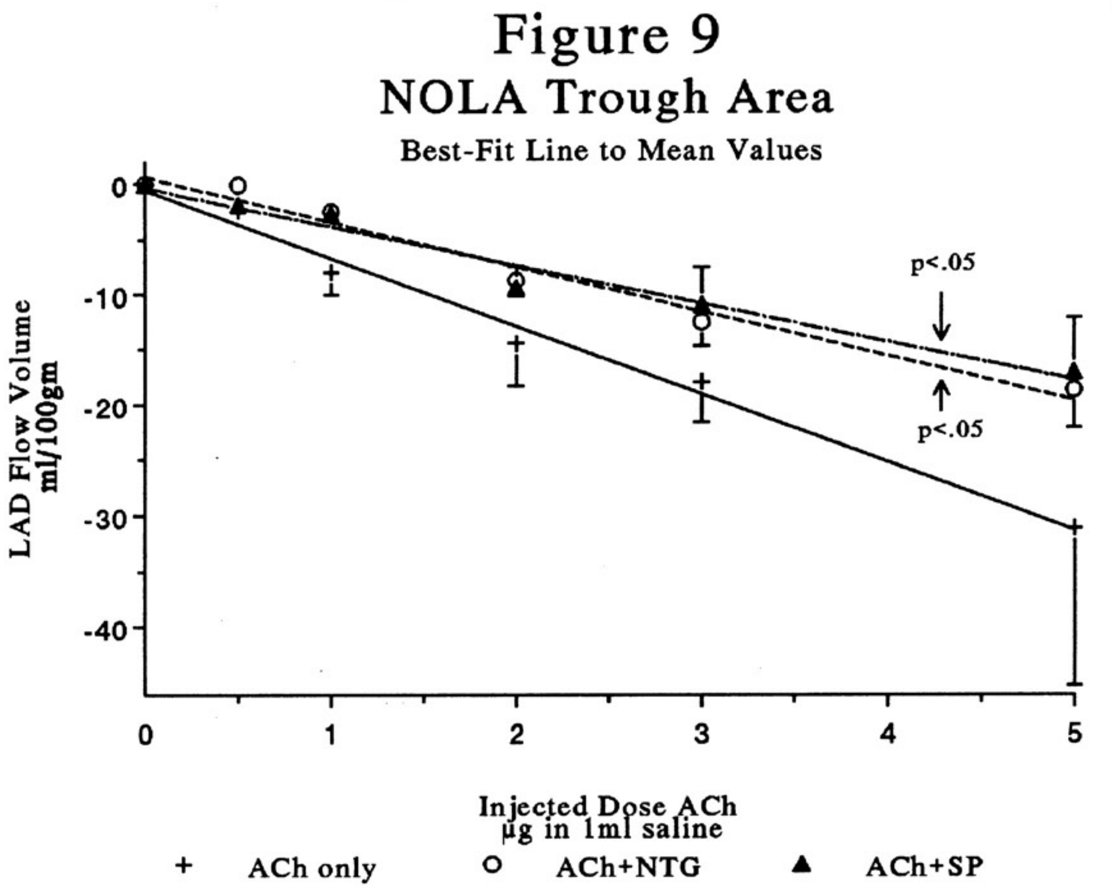

Figure 9. NOLA Trough Area, best-fit line to mean values ± SEM. NOLA = Nω-nitro-L-arginine, LAD = left anterior descending coronary artery, ACh = acetylcholine, NTG = nitroglycerin, SP = substance P. * = p < .05.

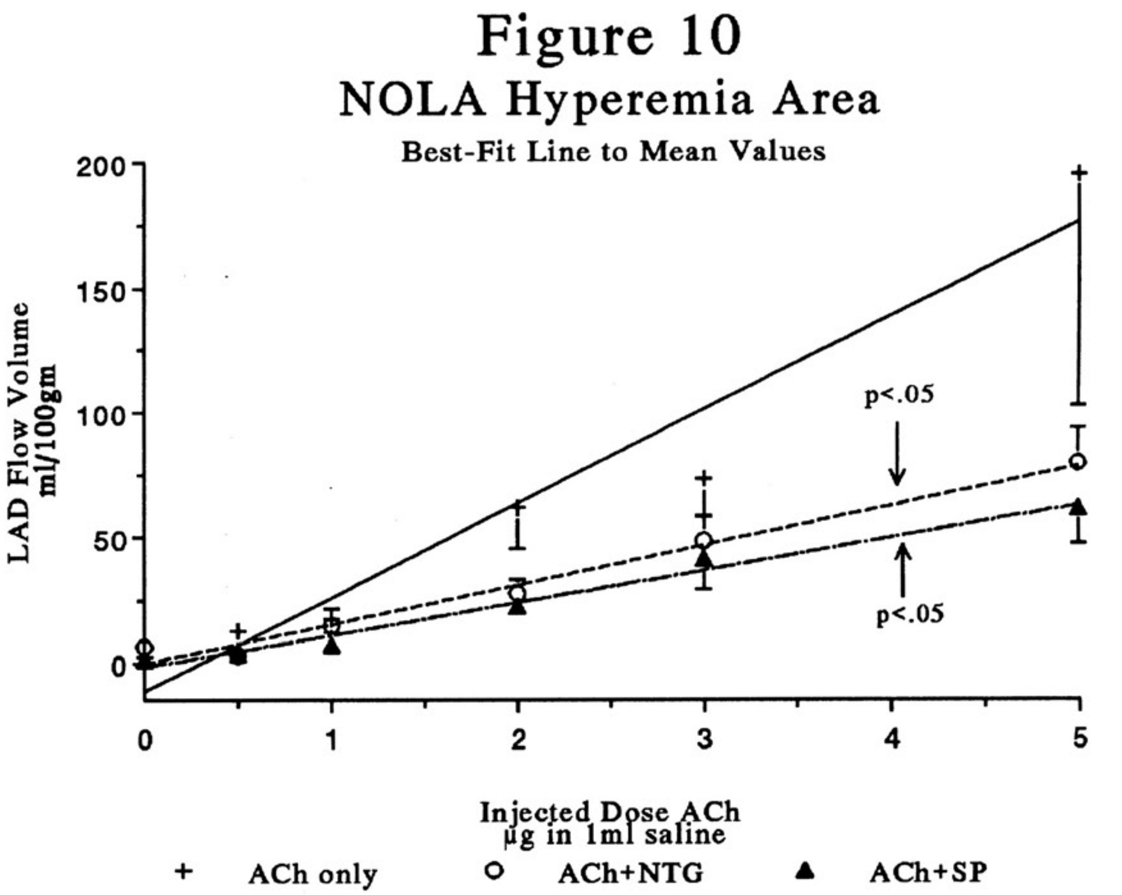

Figure 10. NOLA Hyperemia Area, best-fit line to mean values. Error bars indicate ± SEM. NOLA = N^{ω}-nitro-L-arginine, LAD = left anterior descending coronary artery, ACh = acetylcholine, NTG = nitroglycerin, SP = substance P. Significance indicated at $p < .05$.

Although no significant differences were noted between the NOLA TD trials, examination of the group's means graphed in Figure 11 shows that the Trough values decrease sharply for ACh doses of 1 µg and above and the decline is accentuated for the ACh only curve; a converse but corresponding change similar to that seen in the HD data is illustrated in Figure 8. The best-fit lines of the NOLA Delta mean values are graphed in Figures 12 and 13. Both the NTG+ACh and SP+ACh slopes were significantly different ($p<.05$) from the ACh only slope; no differences were found between the NTG+ACh and SP+ACh slopes or between any of the Y-intercepts.

Between Groups Analyses

Comparisons of the ACh only to NTG+ACh and SP+ACh tests between the Control and NOLA Groups are displayed in Figures 14 through 17. Figure 14, the ACh only vs. NTG+ACh TD means, shows that the NOLA ACh only reached the lowest LAD flows following ACh and that NTG administration partially relieved the loss of flow. The Control data exhibited the same relationship, but with even less loss of flow.

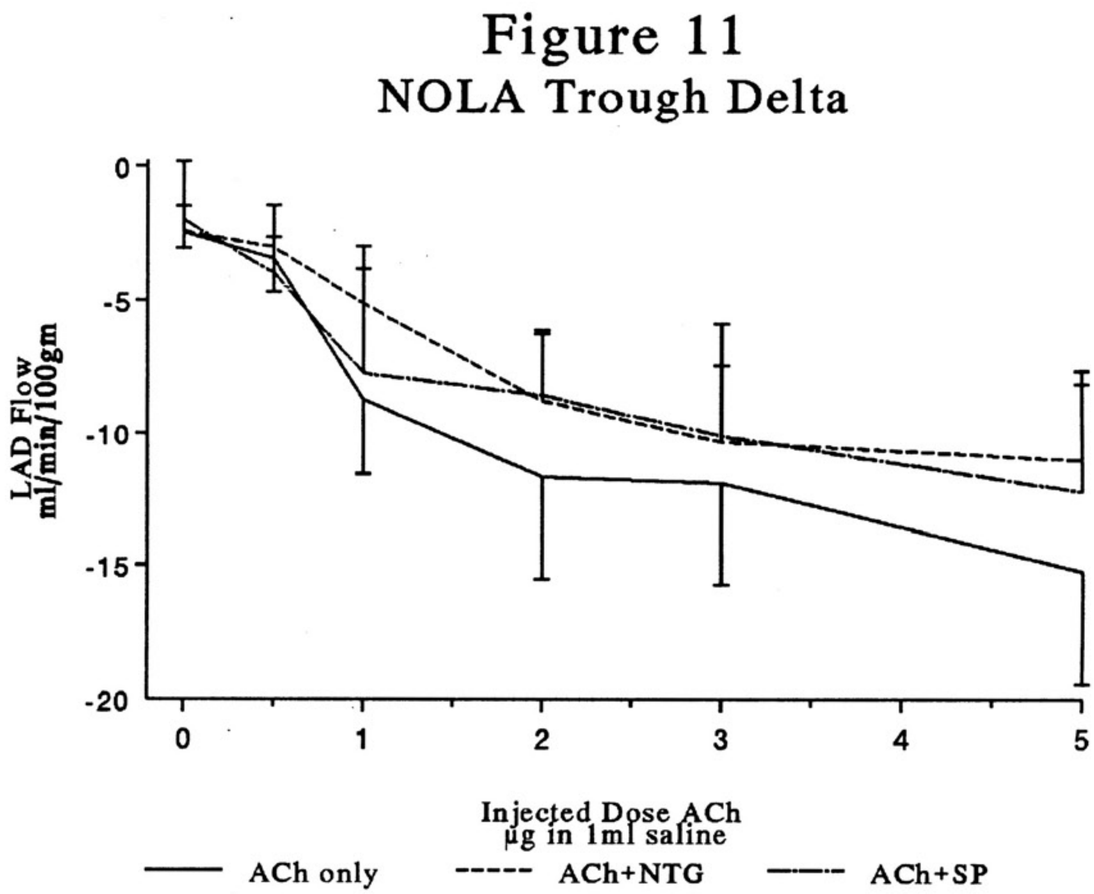

Figure 11. NOLA Trough Delta, mean values ± SEM. NOLA = N^{ω}-nitro-L-arginine, LAD = left anterior descending coronary artery, ACh = acetylcholine, NTG = nitroglycerin, SP = substance P.

Figure 12
NOLA Hyperemia Delta

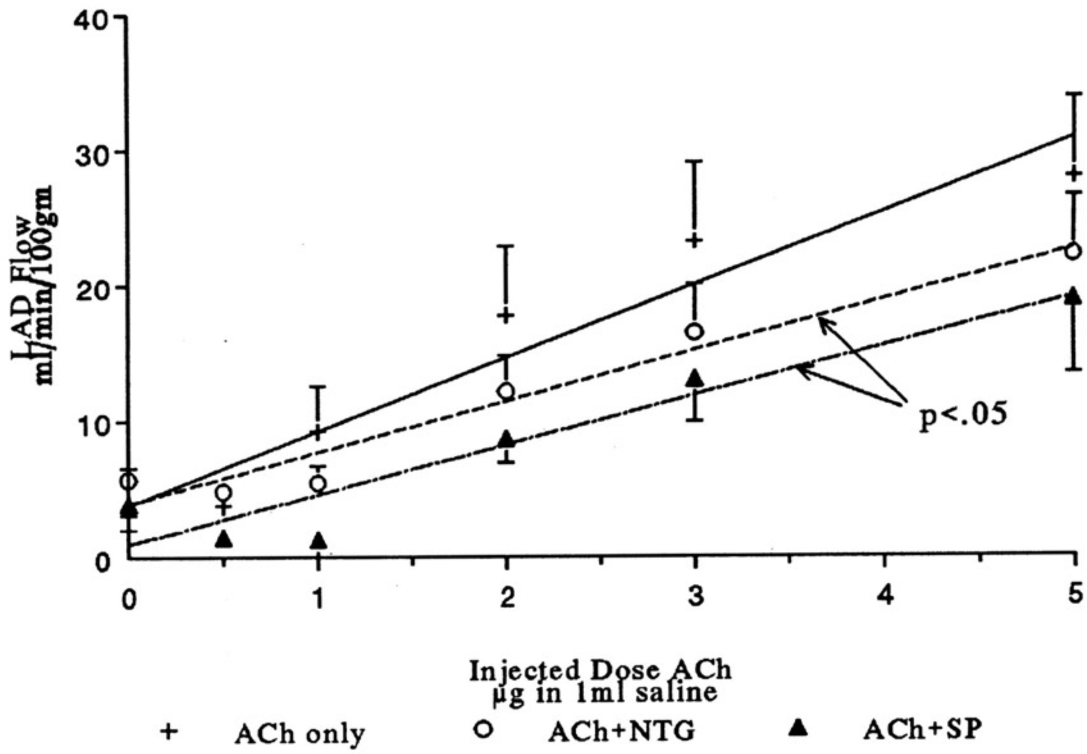

Figure 12. NOLA Hyperemia Delta, best fit to mean values. Error bars indicate ± SEM. NOLA = N^{ω}-nitro-L-arginine, LAD = left anterior descending coronary artery, ACh = acetylcholine, NTG = nitroglycerin, SP = substance P. Significance indicated at $p < .05$.

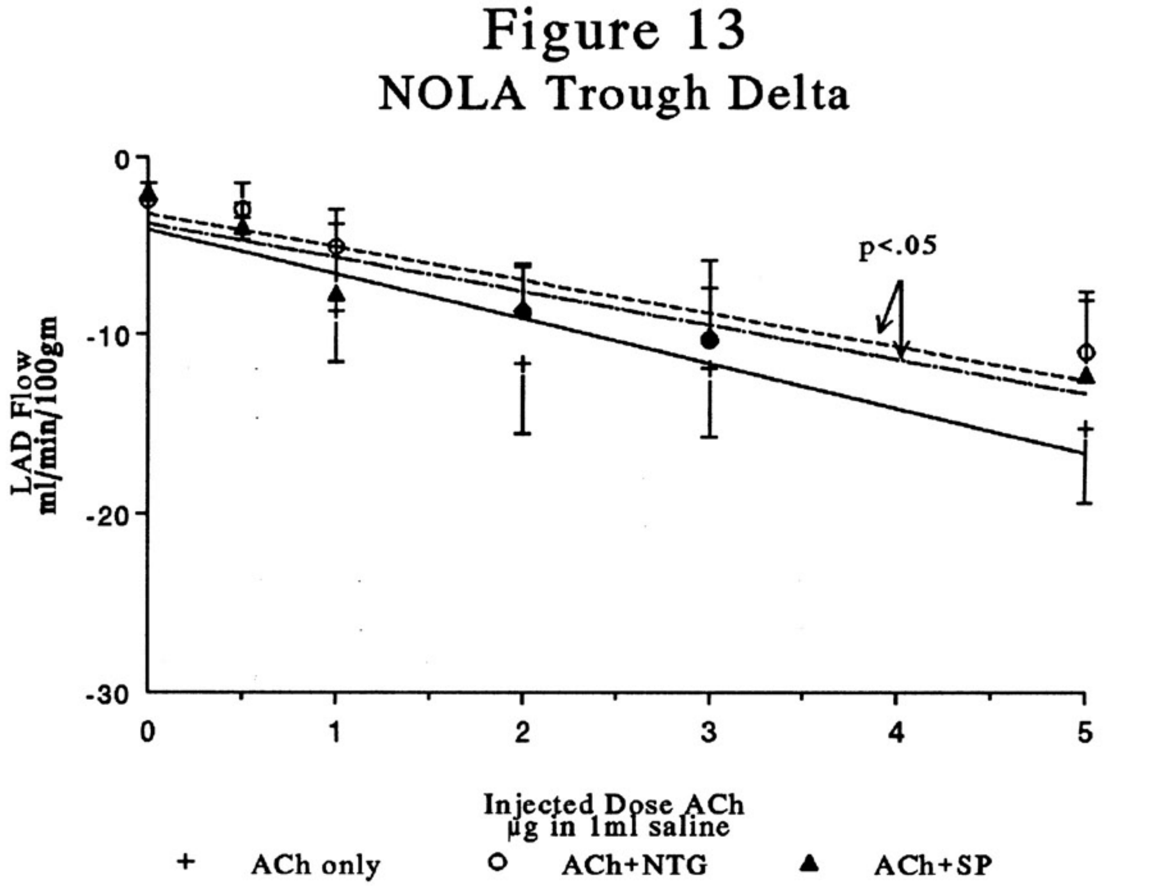

Figure 13. NOLA Trough Delta, best-fit line to mean values. Error bars indicate ± SEM. NOLA = N^{ω}-nitro-L-arginine, LAD = left anterior descending coronary artery, ACh = acetylcholine, NTG = nitroglycerin, SP = substance P. Significance indicated at $p < .05$.

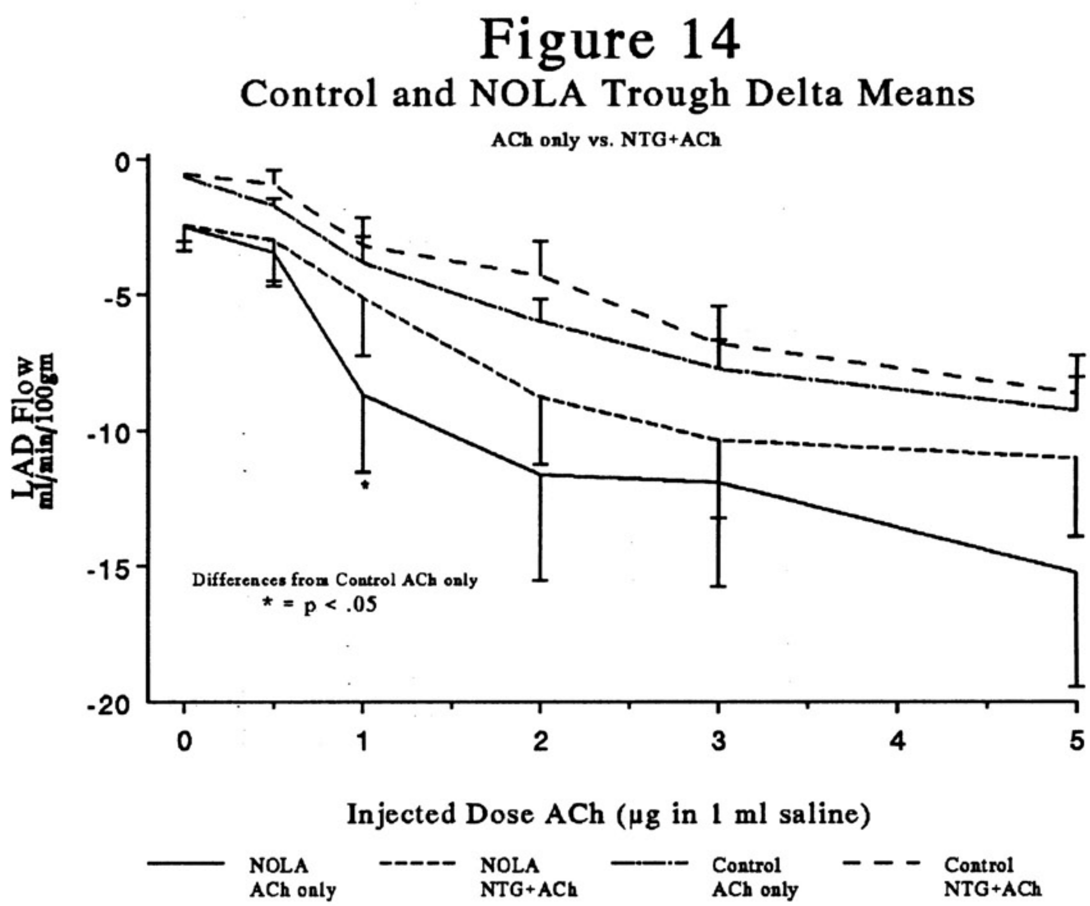

Figure 14. Control and NOLA Trough Delta mean values ± SEM (ACh only versus NTG). NOLA = N^{ω}-nitro-L-arginine, LAD = left anterior descending coronary artery, ACh = acetylcholine, NTG = nitroglycerin. * =p <.05 from Control ACh only.

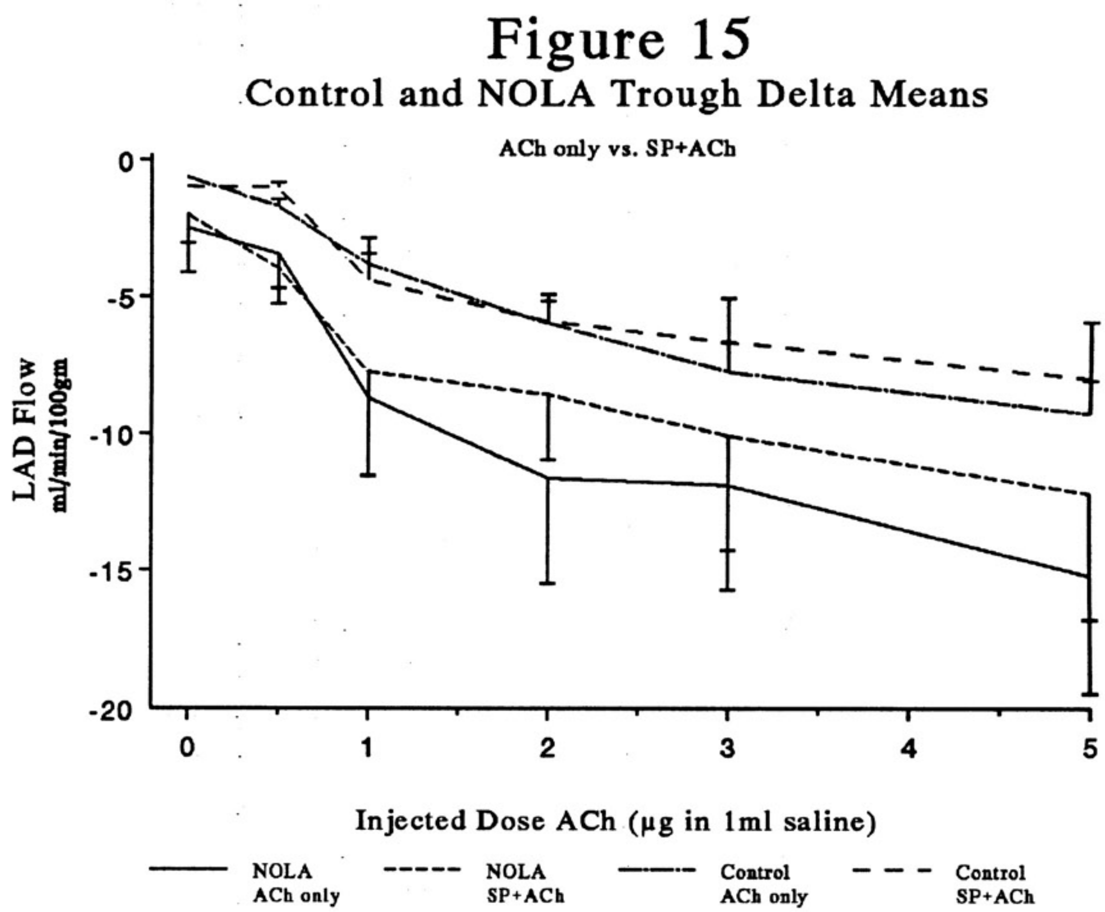

Figure 15. Control and NOLA Trough Delta mean values ± SEM (ACh only versus ACh+SP). NOLA = N^{ω}-nitro-L-arginine, LAD = left anterior descending coronary artery, ACh = acetylcholine, SP = substance P.

The four ACh only vs. NTG+ACh HD means shown in Figure 16 hold the same arrangement seen in the TD data. The NOLA ACh only doses reached the highest flows during hyperemia, followed by the NOLA NTG+ACh, Control ACh only and Control NTG+ACh. The NOLA ACh only data were significantly different from the Control ACh only data for dose of 2 μg and above, while the NOLA ACh only data were different from Control NTG+ACh at 0.5 through 3.0 μg. This relationship is also seen in the ACh only vs. SP+ACh HD means in Figure 17 although the significances are less pronounced.

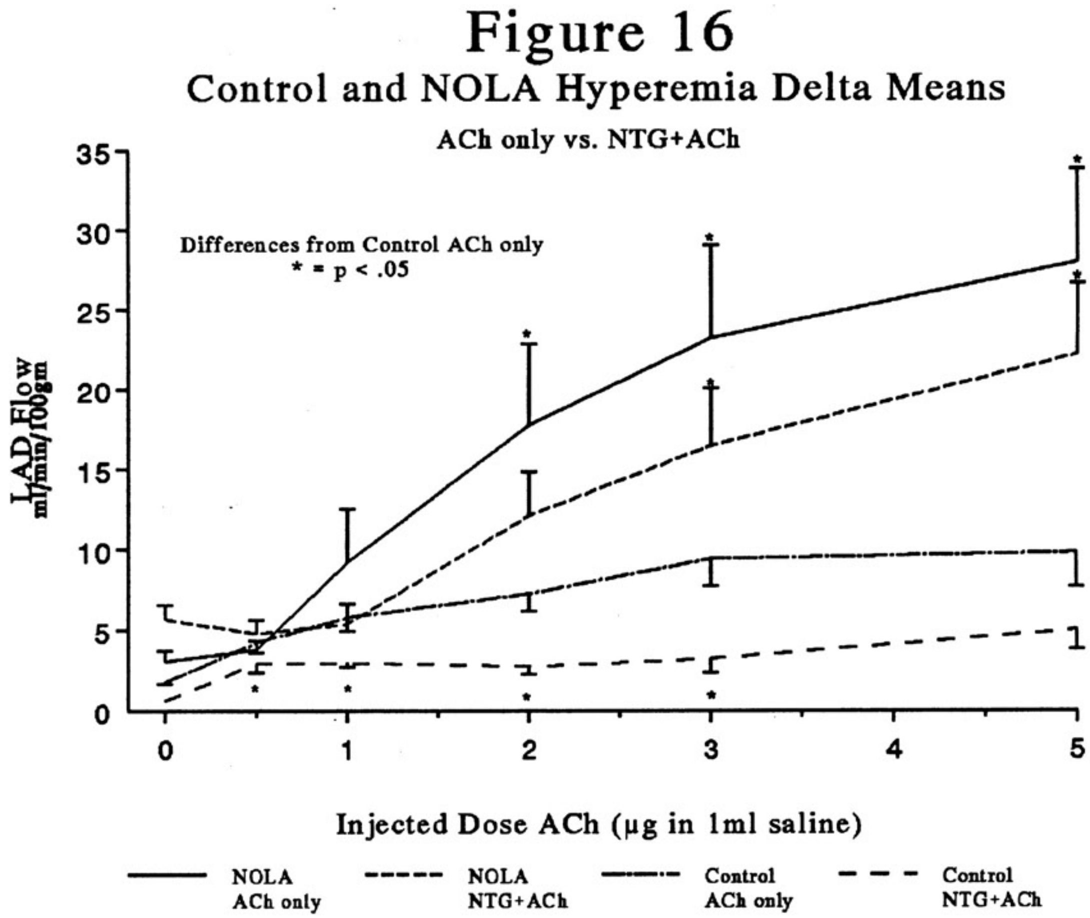

Figure 16. Control and NOLA Hyperemia Delta mean values ± SEM (ACh only versus ACh+NTG). NOLA = N^{ω}-nitro-L-arginine, LAD = left anterior descending coronary artery, ACh = acetylcholine, NTG = nitroglycerin. * = p < .05 from Control ACh only.

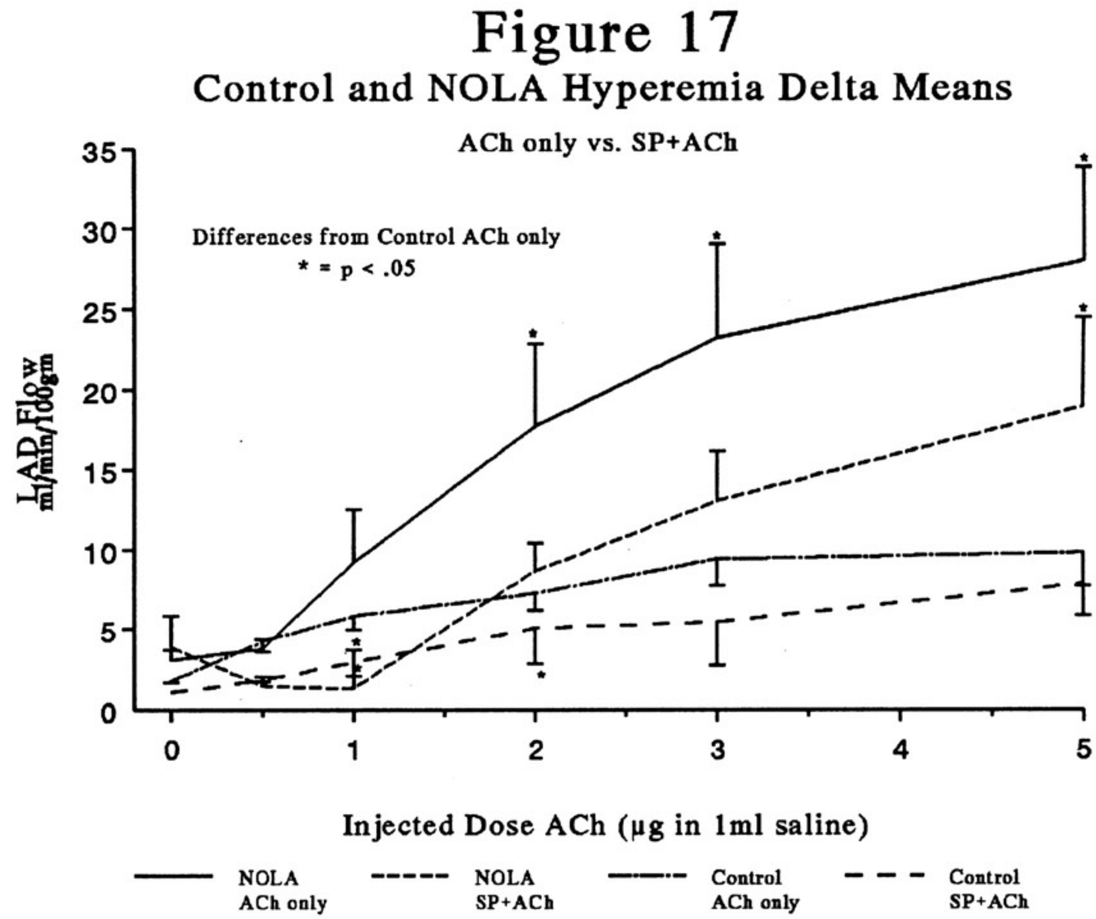

Figure 17. Control and NOLA Hyperemia Delta mean values ± SEM (ACh only versus ACh+SP). NOLA = N^{ω}-nitro-L-arginine, LAD = left anterior descending coronary artery, ACh = acetylcholine, SP = substance P. * = p < .05 from Control ACh only.

DISCUSSION

A number of studies to date concerning the release or blockade of EDRF, especially those using a porcine model, have dealt strictly with *in vitro* conditions. This *in vivo* porcine study has tested endothelial ability to modulate coronary artery flow in the face of a cholinergic vasospastic challenge. Control basal conditions and EDRF blockade by NOLA were compared by testing the endogenous release of EDRF via Substance P and provision of its active moiety exogenously via nitroglycerin. The preliminary phase of the present study entailed the removal of endothelium in order to validate that the endothelium has a prominent role in modulating coronary artery tone.

Denudation Validation

The technique used to evaluate the response to *in vivo* intracoronary ACh has been described. The catheter employed displays a cross-section of the vessel in its immediate vicinity — in this case, one of the larger portions of the coronary conducting vessels. The effect expected at the denuded site upon intracoronary bolus ACh administration was 1) no vasoconstriction prior to denudation, 2) some degree of constriction immediately (*i.e.* within minutes) after denudation, and 3) a maximal vasoconstriction demonstrated several days afterward. The effects actually seen agreed with the prediction with the exception of the immediate

response following denudation. Here, no discernible change in vessel diameter could be determined immediately post-denudation even though histopathology confirmed removal of intimal cells.

This study's immediate-effect prediction was based on the work of Gräser *et. al.*[41,42] and Ito *et. al.*[52]. Gräser described ACh-induced porcine CA vasospasm *in vitro* and established that it is independent of the presence of endothelium, *i.e.* that ACh acts directly on the vascular smooth muscle (VSM) cell. Ito[52] showed that ACh mobilized stored calcium, an effect blocked by atropine, and concluded muscarinic receptors exist on porcine vascular smooth muscle cells that liberate calcium which initiates VSM contraction. The muscarinic receptors responsible for contraction were later characterized by van Charldorp and van Zwieten to be M3 and M4 subtypes[98].

Even though the presence of these receptors has been established, other authors have noted a lack of denuded vessel reactivity to ACh in dilated areas as was seen in this study. Lam *et. al.*[58], and prior to him Cragg *et. al.*[23], described non-reactive 'vessel paralysis' in denuded areas. Lam *et. al.* felt the effect was "probably related to severe injury to the smooth muscle cells in the dilated region, which results in histologic evidence of necrosis by 24 hr, and thus an inability to respond"[58]. Infrequent necrosis was seen post-mortem in some of this work's experimental animals which could account for the lack of reactivity immediately post-denudation, but varying degrees of necrosis were seen and all animals were non-reactive immediately post-denudation.

A second possibility is that balloon denudation overstretches vascular smooth muscle causing the 'disconnection' of VSM actin-myosin crossbridge linkages which could temporarily inactivate contractile ability. Indirect evidence supporting this mechanical dissociation comes from a work that used an extracorporeal distilled water circuit to osmotically lyse endothelial cells, not an expansive (and therefore VSM-stretching) balloon catheter[88]. In this study, Schipke *et. al.* found that canine coronary arteries so denuded contracted immediately in response to ACh, not hours later.

ACh contractile effects seen after denudation in this study began within 24 hours of denudation and achieved maximal effect 7 to 10 days afterward. This agrees with other authors who have noted that when the endothelium is damaged, ACh leads to either a loss of vasodilatory capability or vasoconstriction that is nearly species-independent. This 'paradoxical vasoconstriction' (as it has been termed[61]) has been demonstrated acutely in human[78,80], monkey[45], canine[3,75,88] and porcine[5] vessels. The general concensus among authors reviewed is that denudation removes dilatory capability as well as exposing VSM cells to the vessel lumen and blood-borne agonists.

<u>Effect of Dilator Infusion and ACh Injection on Baseline</u>

Examination of Figure 4 shows that for basal coronary flow values there is no difference between the depth of the trough and the peak of the hyperemic episode following it. In fact, the two curves appear to be mirror imaged about a

common baseline. Dilator infusion, on the other hand, demonstrated some differences in effect that were not expected. NTG infusion was expected to, and did, increase coronary flow, but SP infusion decreased flow. Although neither change was found to be statistically significant, the decrease in flow due to SP infusion was noteworthy and is discussed further in the Comparison of Control Group to NOLA Group section below.

Control Study

This study compared the effect of an ACh Dose Response Curve given in a non-treated state (Control ACh only) to the same DRC given during infusion of an exogenous NO source, nitroglycerin (Control NTG+ACh), and an endothelial or endogenous source, Substance P (Control SP+ACh). The effect of ACh on coronary flow in an *in vivo* pig model was established by Cowan and McKenzie, who showed that ACh preferentially constricts porcine coronary vasculature[21]. The earlier work of Gräser *et. al.*[41,42] *in vitro* had already established that ACh contracts porcine coronary artery VSM directly, *i.e.* without endothelial involvement. Cowan found that a bolus dose of 2.5μg ACh given intracoronary through an infusion catheter reduced conductance by 78.2% and flow by 77%[21]. His work in open-chest swine formed the basis for the use of ACh as a vasospastic agonist in this study.

The expected effects of ACh were a dose-dependent decrease in CA flow (as discussed above) followed by a dose-dependent hyperemic phase known by

the term 'reactive hyperemia'. Olsson's review of myocardial reactive hyperemia indicates that the coronary flow peak of the hyperemic response that follows myocardial ischemia may be 5 to 6 times that of control values[81]. Of the hypotheses he discusses to account for this, the production of a vasodilatory metabolite during an ischemic episode appears the strongest. The release of adenosine during myocardial ischemia and the subsequent vasodilatory hyperemia it produces have been validated[67].

The infusion of NTG and SP were expected to blunt the effects of ACh through their vasodilatory capability. The Control Trough NTG+ACh and SP+ACh values were found to be significantly different from Control ACh only in the Delta data set only at the 0.5µg dose. Lack of Trough data significance at higher ACh doses is contrary to results seen in the Hyperemia data. This is attributed to the inherent lack of sensitivity seen when measuring decreasing blood flows. Absolute flows in the LADCA rapidly fall to or near zero in response to ACh doses of 1µg and above regardless of the presence of a vasodilator. Since blood flow minimums can never fall below zero, no significance can be found at ACh doses that suppress flow to this minimum. The Hyperemia data in contrast is not constrained to a fixed limit and responds in proportion to the ACh dose employed.

Regression analysis of the Trough Area slopes demonstrated that NTG+ACh and SP+ACh slopes were both different from ACh only at $p<.05$. The conclusion drawn from this information, and the fact that Y-intercepts did not differ, was that loss of flow is preferentially relieved at higher doses. In other words, the increase

in flow provided by a NO source, whether endogenous or exogenous, is of less benefit (or is just less needed) during a low-grade vasospastic event than during a severe ischemic episode.

The reduction in TA caused by the vasodilators NTG and SP is paralleled by reduction in the follow-on Hyperemia Area. The Control HA's graphed in Figure 6 provide an excellent example of the effects of both endogenous and exogenous NO generators on vasospasm. Large differences exist between the ACh only and NTG+ACh curves, less so for ACh only and SP+ACh. The conclusion that can be drawn here is that the addition of NO sources result in less loss of flow (a higher Trough) and a concomitant reduction in the 'payback' required following the ischemia (a lower Hyperemia). The loss of this innate vasodilatory capability, whether through endothelial damage or chemical blockade, should be expected to have an adverse effect on flow maintenance to the perfused area downstream of a vasospastic locus.

NOLA-blockade Study

This study compared the effect of an ACh Dose Response Curve given during nitric oxide blockade by NOLA in a non-treated state (NOLA ACh only) to the same DRC given during nitroglycerin (NOLA NTG+ACh) and Substance P (NOLA SP+ACh) infusions. Arginine analogues such as NOLA (N^{ω}-nitro-L-arginine) inhibit the activity of endothelial NO synthase, the enzyme that catalyzes the formation of endogenous endothelial NO.[68] Blockade of NO in this study was

expected to negate the vasodilatory effect of SP seen in the Control group, rendering it ineffective in attenuating acetylcholine's impact. The results from the NOLA SP+ACh group should then approximate that of the NOLA ACh only group.

Analysis of the NOLA Hyperemia data established differences for the NOLA HA NTG+ACh and SP+ACh groups ($p<.05$) from the ACh only group. Figure 9 and 10 graphically illustrate the effect of NOLA blockade on exogenous and endogenous NO sources. These graphs disagree with the earlier prediction that the exogenous NO source would continue to be valid in the presence of blockade of endogenous NO production; further discussion of this discrepancy continues in the next section.

Comparison of Control Group to NOLA Group

Graphic comparison of the Control and NOLA-blocked TD means can be found in Figures 14 and 15. These graphs compare between-group events to the Control ACh only condition. Statistically, no significant difference is seen here that allows ready conclusions to be drawn, but the arrangement of the data for the most part agrees with expected results. The NTG+ACh data in Figure 14 shows less negative (trough) flows for both Control and NOLA when compared to their respective ACh only plot, indicating at least partial relief from the vasospasm caused by ACh. The exception to expected results is the NOLA SP+ACh data seen in Figure 15. Close agreement between the NOLA ACh only and NOLA SP+ACh values was expected because the NO synthase inhibition by NOLA

should have blocked any endogenous vasodilation by Substance P. By observation it can be seen, however, that the inhibition fails for the higher doses of ACh (2,3, and 5µg), although it is not statistically significant.

No ready answer is available to explain this discrepancy, but Bény and Brunet provided a possible explanation in their work with pig coronary arteries *in vitro*.[4] They concluded from work with NO and NTG that these two compounds did not account for all the endothelially-mediated vasodilatory characteristics. In particular, NO and NTG do not hyperpolarize smooth muscle cells, nor interfere with the hyperpolarization due to EDRF released by SP. According to their work other factors distinct from those mentioned must be at work as well to account for the vasodilation. Christie and Lewis[15] also found that responses to EDRF release differed with regard to both the artery type and the animal, indicating that different sensitivities to NO exist.

Another possible explanation for the NOLA Trough SP discrepancy may be that 4 mg/kg NOLA was insufficient to completely block NO release by SP, but there is evidence to discount this. An *in vivo* rat study[17] used similar concentrations (0.1 to 10 mg/kg) with adequate effect, and Zambetis *et. al.* found little difference between 5, 15, and 60 mg/kg of NOLA when given to rats.[101] Mean arterial pressure, the best indicator of NO synthase blockade, increased in the NOLA-blocked animals of the present study by 8.6±.96 mm/Hg, indicative of sufficient activity.[101]

The HD Means graphed in Figures 16 and 17 parallel the responses seen in the TD data. The relationships between the data in both graphs are as predicted with the exception of the NOLA SP+ACh seen in Figure 17. Again, the argument put forth by Bény and Brunet[4] would offer an explanation for the vasodilation seen in the NOLA SP+ACh curve. The remainder of the curves lie as predicted earlier, with the vasodilation of exogenous and endogenous NO sources limiting the trough and attenuating the hyperemia caused by a cholinergic vasospastic challenge.

In conclusion, this study has examined the interactions of endogenous and exogenous sources of nitric oxide, a vascular smooth muscle dilator, with acetylcholine, the muscarinic vasospastic agonist, in an *in vivo*, open-chest porcine model. The findings described here validate the endothelial source of NO and detail some of the hemodynamic effects of its blockade, as well as vasospastic challenges applied against it. Acetylcholine produced initial dose-dependent decreases in coronary blood flow, immediately followed by a dose-dependent hyperemia. Nitroglycerin, an exogenous source of nitric oxide, partially relieved both the decrease in flow experienced during the trough and the peak of the hyperemic flow. Substance P, a tachykinin that liberates NO from the endothelial cell, was capable of similar effects that could be blocked by an L-arginine analogue, N^{ω}-nitro-L-arginine. The sum of this work adds to the body of literature concerned with endothelial functions and mediation of coronary flow.

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